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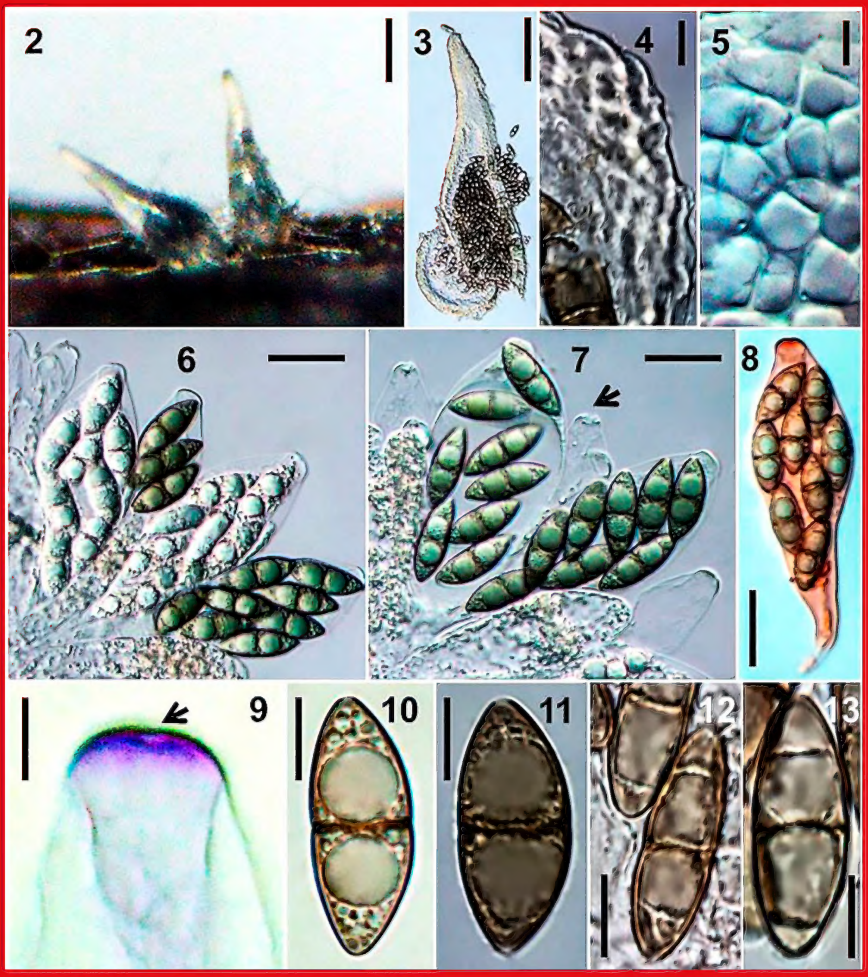

Phaeonectriella alba sp. nov.
(Abdel-Azziz & al.— FIGS 2–13, p. 603)

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was issued on October 29, 2018

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The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this quarter.

Ilgaz Akata
Timothy J. Baroni
Rafael F. Castañeda-Ruiz
Vagner Gularte Cortez
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Pradeep K. Divakar
Nils Hallenberg
Genevieve Gates
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Marisol Sanchez-Garcia
Hana Ševčíková
B.M. Sharma
Steven L. Stephenson
Yong Wang
Ming Ye
Xiu-Guo Zhang

From the Editors

Good-bye Mycobiota / Herbaria; Hello Funga / Fungaria? Many of you have undoubtedly found yourselves adding "I study fungi" after facing blank looks brought on by your answering "I am a mycologist" when asked what it is you do. As it is obvious that the non-scientific public recognizes 'fungi' better than 'myco-whatever,' we draw your attention to a very convincing paper by Kuhar & al. [IMA Fungus. 2018. 9(2): 71–74] proposing the "Delimitation of Funga as a valid term for the diversity of fungal communities: the Fauna, Flora & Funga proposal (FF&F)."

Recognizing that alliteration is innately pleasing, Kuhar & al. acknowledge that "the term Mycobiota has also been applied to the fungal components of a community and can be considered a synonym of Funga. Although entirely correct, we think that the educational and governmental sectors would better accept a Latinized term in accordance with Fauna and Flora." Having early rejected 'mycoflora' and then spent considerable time mulling over what to call our hosted 'annotated species lists' (shortened to 'weblists')—and despite having finally settled last year on 'mycobiota'—we believe that 'Funga' (and its plural 'Fungae') appeals and are thinking of adopting that word in the future.

In his "Funga and fungarium" Hawksworth (2010. IMA Fungus 1: 9) threw down the gauntlet asking mycologists to assert "their independence from botanists, (other) microbiologists, and zoologists as a part of the long road to increased recognition amongst the life sciences." He also adopted Spooner & Cannon's term ('fungarium' (pl. 'fungaria') "as a counterpart to 'herbarium' (a collection of dried plants)," noting that fungarium surely merited wider adoption.

Although we do suggest using (and will accept) Funga and fungaria in future papers, be assured that we shall not be calling this esteemed publication Fungataxon any time soon!

New requirement for type designation identifiers—Pay heed to the following important information from Nomenclature Editor Pennycook:

"Authors who intend to designate new lectotypes, neotypes, or epitypes are advised of a new requirement. On 1 January 2019, a new article of the nomenclatural code [ICN (Shenzhen) Article F.5.4] came into force, requiring designations of all new types (except holotypes) to include an identifier number issued by one of the recognised repositories (Index Fungorum, MycoBank, or Fungal Names). A type designation published without an identifier will have no priority against any future designation of a type for the same taxon."

The ICN <https://www.iapt-taxon.org/nomen/pages/main/art_f5.html> states: "F.5.4. For purposes of priority (Art. 9.19, 9.20, and 10.5), designation of a type, on or after 1 January 2019, of the name of an organism treated as a fungus under this Code (Pre. 8), is achieved only if an identifier issued by a recognized repository (Art. F.5.3) is cited.

"Note 3. Art. F.5.4 applies only to the designation of lectotypes (and their equivalents under Art. 10), neotypes, and epitypes; it does not apply to the designation of a holotype when publishing the name of a new taxon, for which see Art. F.5.2."

Are FN, MB, and IF necessary for nomenclatural identifiers? We are pleased to report that the three nomenclatural repositories (Fungal Names, MycoBank, and IndexFungorum) are working together smoothly (at least for new additions), placing fungal nomenclature far ahead of others. No longer must we toil in dusty library stacks tracking down early historical names to suss out priorities; these days we are far less likely to commit the unpardonable sin of illegitimately proposing a new taxon with a name already used in a particular genus.

Recently the Nomenclature Committee for Fungi discussed whether alpha-prefixes designating the issuing depository are required for valid publication of a new name.

Strictly speaking, no. Numbers have been assigned in 'batches' to each database, making each number unique and thus not needing a data-base reference to validate the name to which it has been assigned.

However, Mycotaxon will continue to require FN/IF/MB prefixes as part of the identifiers, as it considerably eases finding both nomenclatural and taxonomic information so necessary to our readers.

The delayed Mycotaxon 133(4) contains 18 papers by 63 authors (representing 13 countries) and reviewed by 34 expert reviewers.

Within its pages are 12 species new to science representing *Cladosporium, Gyalecta, Rosellinia,* and *Soleella* from China; *Escovopsis* from Argentina; *Phaeonectriella* from Egypt; and *Sistotrema* from India.

In addition to range extensions and/or new hosts for previously named taxa, we also offer a newly established synonymy in *Conidiobolus* and validation of five Indian corticioid species.

We sincerely apologize for delays caused by the Editor-in-Chief's unanticipated but continual travails with water leaks, remodels, holiday celebrations, and the January 2019 two-week cold virus (among other indignities). Eternally optimistic, we hope to be back on schedule, delivering our next issue on time, much thicker, and in March!

Warm regards,

Lorelei L. Norvell (*Editor-in-Chief*)
Shaun R. Pennycook (*Nomenclature Editor*)
3 February 2019

2019 Mycotaxon submission procedure

Prospective Mycotaxon authors should download the Mycotaxon 2019 guide, review & submission forms, and Mycotaxon sample manuscript by clicking the 'file download page' link on our Instructions to Authors page before preparing their manuscript. This page summarizes our '4-step' submission process.

1—Peer review: Authors first contact two (for journal papers) or three (for mycobiota / fungae) peer reviewers before sending them formatted text & illustration files and the appropriate 2019 Mycotaxon journal or weblist reviewer comment form. Experts return revisions & comments to both the *Editor-in-Chief* <editor@mycotaxon.com> and authors. The authors correct their files before submitting their manuscript to the *Nomenclature Editor*.

2—Nomenclatural review: Email all **error-free** text and illustration files to the *Nomenclature Editor* <PennycookS@LandcareResearch.co.nz>. Place **first author surname** + **genus** + 'Mycotaxon' on the subject line; list title & coauthors and peer reviewer names & Email addresses in the message. The Nomenclature Editor will (i) reply immediately with the accession number and (2) return his notes with suggested revisions to the author(s) and *Editor-in-Chief* after a few weeks.

3—Final submission: Authors should consult experts and thoroughly revise and proof-read manuscripts to prepare error-free text and image files ready for immediate publication. **Label** the final submission email to the *Editor-in-Chief* <editor@mycotaxon.com> and **all** files & forms with **accession number;** attach the **(i)** 2019 submission form, **(ii)** labeled text files, **(iii)** labeled jpg files, and [required] **(iv)** FN, IF, or MB identification verification for each new name or typification. The *Editor-in-Chief* usually contacts all coauthors and expert reviewers within two weeks of final submission, but please wait at least 14 days before sending a follow-up query (without attachments).

4—Final editorial review: Files with errors will be rejected or returned for revision. The *Editor-in-Chief* conducts a final grammatical and scientific review and returns her editorial revisions to all expert reviewers and coauthors for final author approval. Author-approved files are placed in the publication queue.

The PDF proof and bibliographic & nomenclatural index entries will be sent to all coauthors for final inspection. After PDF processing, the *Editor-in-Chief* corrects only PDF editorial/conversion and index entry errors; corrections of all other errors are listed in the Errata of a subsequent issue for no charge. Authors should arrange payment of page charges and optional open access fees with the *Business Manager* <subscriptions@mycotaxon.com> at this time.

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New reports of the plant pathogens *Aecidium viburni* and *Erysiphe viburni* from Pakistan

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Abstract—The rust pathogen *Aecidium viburni* and the powdery mildew *Erysiphe viburni* were isolated from leaves of *Viburnum grandiflorum*, establishing new records for Pakistan mycobiota.

Key words —Abbottabad, *Ascomycota*, *Uredinales*

Introduction

Viburnum grandiflorum Wall. ex DC. (grand Viburnum), belonging to *Adoxaceae*, is one of the most common shrubs in the Himalayas between 1500–3000 m altitude. The flowers often bloom soon after the leaves have fallen in November and continue to appear until June. While the flowers are sweet-scented, the leaves emit a bad smell when bruised. This plant is considered economically important because of its sweet edible fruits. Seed juice is used for treating typhoid and whooping cough (Malik & al. 2011). Ripe fruits are eaten raw and stems are used as fuel (Rana & al. 2014). Leaves and fruits are given to cattle for constipation and fruits are used to treat stomachache. The bark is used to make ropes (Amjad & Arshad 2014). This economically important plant is considered to be stressed by various fungal pathogens. In the present study, we identified two fungal species infecting *V. grandiflorum: Aecidium viburni* and *Erysiphe viburni*. Both species represent new records for Pakistan.

Materials & methods

During field surveys of Himalayan forests in Abbottabad and Swat districts, Pakistan, leaves of *Viburnum grandiflorum* were found infected with two fungal pathogens. The infected specimens were preserved and photographed both in the field and under a Meiji EMZ-5TR stereomicroscope. Free hand sections were cut, and scrape mounts of infected portions were observed. Twenty–twenty-five spores from each spore state were examined under the Nikon YS 100 compound microscope and measured at 400× using a ScopeImage 9.0(X5).exe. Sections and spores of the rust fungus and the powdery mildew hyphae, chasmothecia, asci, and ascospores were photographed with a Digiporo-Labomed camera and illustrated using a Leitz camera lucida. Spore surface ornamentation was observed using Scanning Electron Microscopy (SEM) with EDX. For correct identifications we consulted experts, previously published literature (Braun & Cook 2012; Hiratsuka & al. 1992), the U.S. National Fungus Collections Literature Database (https://nt.ars-grin.gov/), and Index Fungorum (www.indexfungorum.org/). Field specimens were deposited in the Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Taxonomy

Aecidium viburni Henn. & Shirai,
Bot. Jahrb. Syst. 28(2): 265 (1900) PLS 1, 3A,B, 4A–C

Spermogonia adaxial, group 1, type 1, yellowish brown, 86–113 µm diam. Aecia adaxial, densely gregarious, brown to dark brown lesions, irregular. Aeciospores globose, subglobose or ellipsoid, hyaline to subhyaline, 18–23 × 20–25 µm, wall densely verrucose, 2.5–3.6 µm thick. Peridial cells hyaline, angular to nearly rhomboid, 16–23 × 21–25 µm, wall verrucose, ≤2 µm thick.

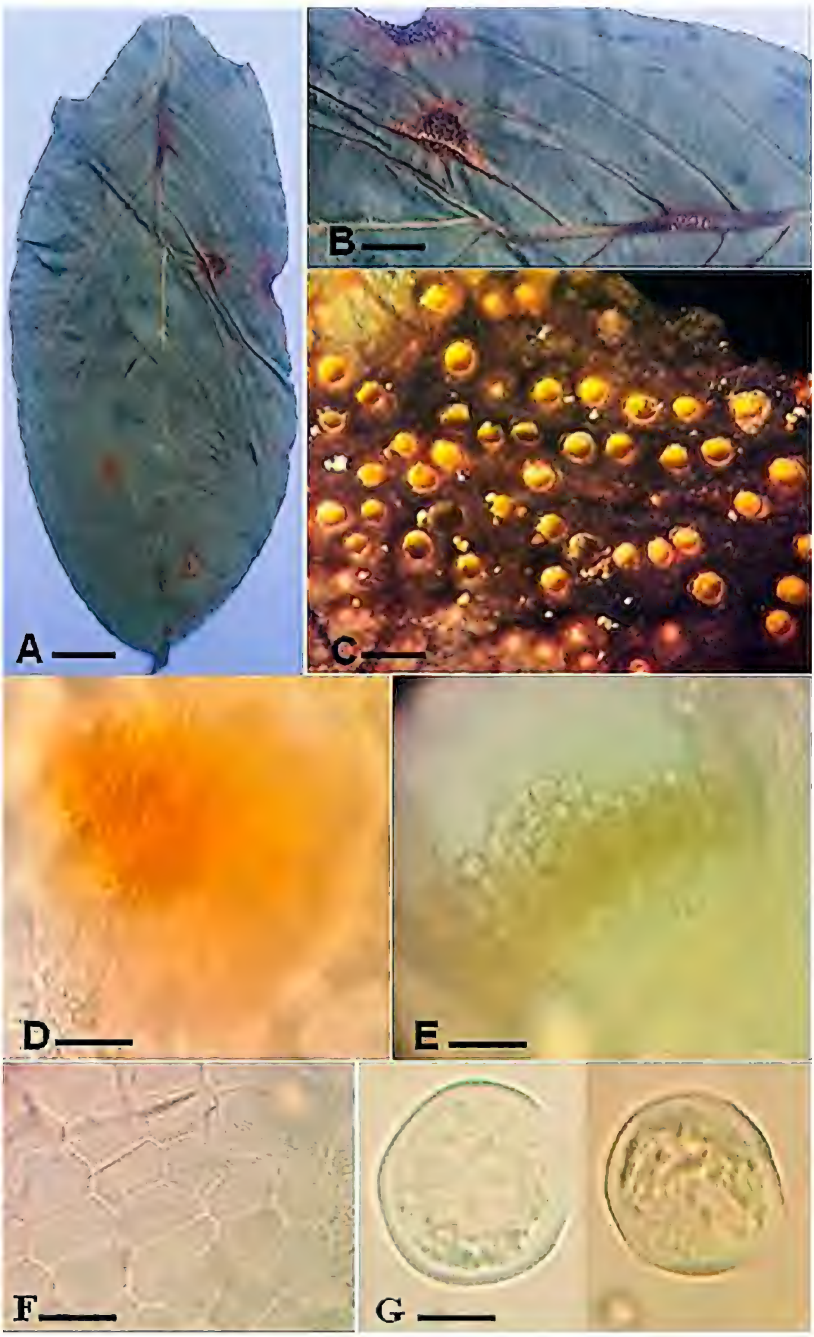
SPECIMEN EXAMINED: **PAKISTAN**, **KHYBER PAKHTUNKHWA**, **Abbottabad district**, Khanspur, at 2250 m asl, on living leaves of *Viburnum grandiflorum*, stages 0 + I, 1 July 2015, A. Ishaq KP-50 (LAH-AM20002).

COMMENTS—Previously reported on various *Viburnum* species from China, Japan, Korea, and Russia (Siberia) (Farr & Rossman 2017). *Aecidium viburni* is reported for the first time from Pakistan, and this is the first record of *Viburnum* as a rust host in Pakistan.

Erysiphe viburni Duby, Bot. Gall. 2: 872 (1830) PLS 2, 3C–G, 4D–F

Mycelium amphigenous, effuse or in patches; hyphae hyaline, smooth; chasmothecium scattered to almost gregarious, globose, dark brown, 90–115 µm

PLATE 1. ***Aecidium viburni*** (LAH-AM20002). A–C. Infected host plant. *Viburnum grandiflorum*; D. Spermogonium; E. Aecium with chains of aeciospores; F. Peridial cells; G. Aeciospores. Scale bars: A = 0.8 cm; B = 1 cm; C = 1 mm; D, E = 20 mm; F = 13.5 µm; G = 9 µm.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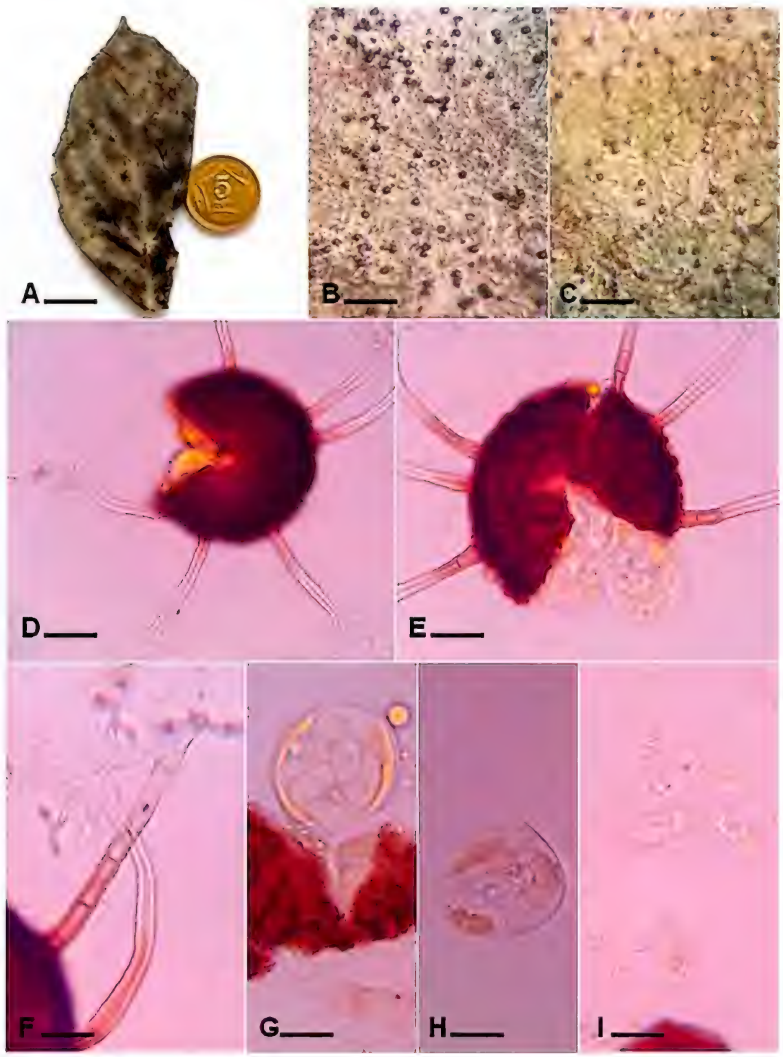

Plate 2. ***Erysiphe viburni*** (LAH-E0001). A–C. Infected host plant *Viburnum grandiflorum*; D. Chasmothecium with dichotomous appendages; E. Broken chasmothecium with asci; F. Apical part of appendages; G, H. Ascus with ascospores; I. Mature ascospores. Scale bars: A = 1.7 cm; B, C = 1 mm; D = 28.5 µm; E = 24 µm; F, G = 18 µm; H, I = 19 µm.

diam.; peridial cells not conspicuous, small, irregularly shaped; appendages 6–8 in number, equatorial, stiff, mostly curved, 0.8–1.2 times as long as the chasmothecial diameter, about 7–9 µm wide, dichotomously branched,

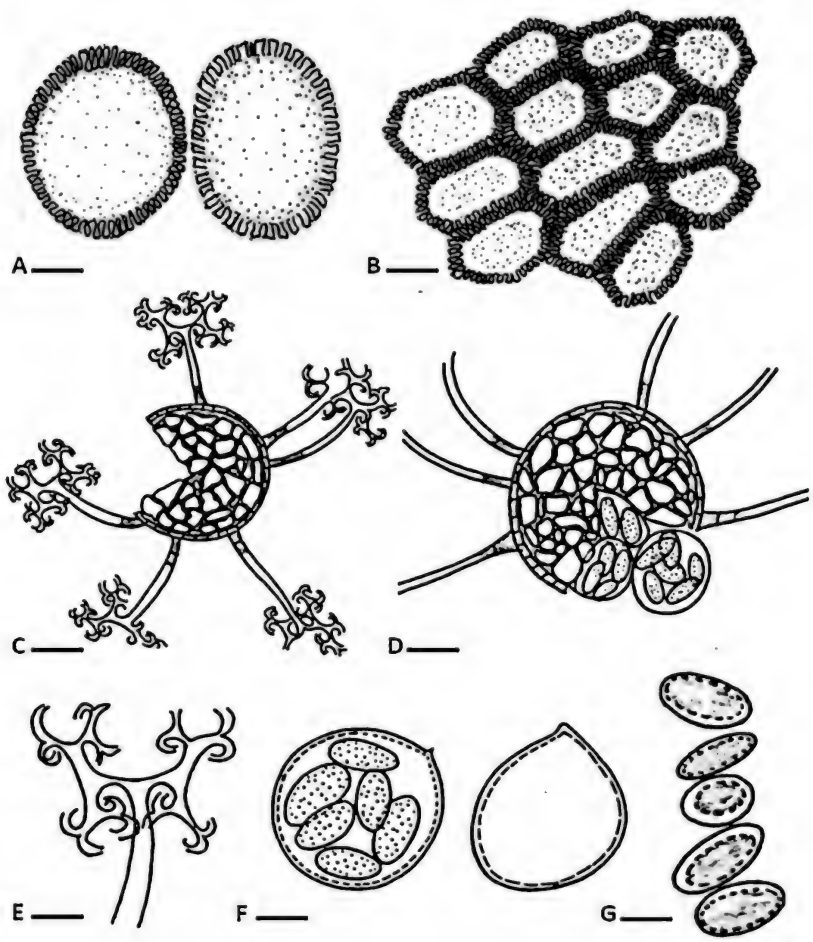

Plate 3. ***Aecidium viburni*** (LAH-AM20002). A. Mature aeciospores, B. Peridial cells. ***Erysiphe viburni*** (LAH-E0001). C. Chasmothecium with dichotomous appendages, D. Broken chasmothecium with asci, E. Dichotomous appendage showing curved tips, F. Asci with ascospores, G. Ascospores. Scale bars: A = 6 μm; B = 9 μm; C = 40 μm; D = 27.5 μm; E = 15 μm; F = 16.5 μm; G = 8 μm.

aseptate or with 1–2 septa at base, hyaline but pigmented at base, walls rough, moderately thickened throughout or thin above and thick towards base; at apex branched 4–5 times, regularly and densely dichotomously, tips distinctly recurved; asci 1–3, globose to ovoid, 43–61 × 37–53 μm, sessile

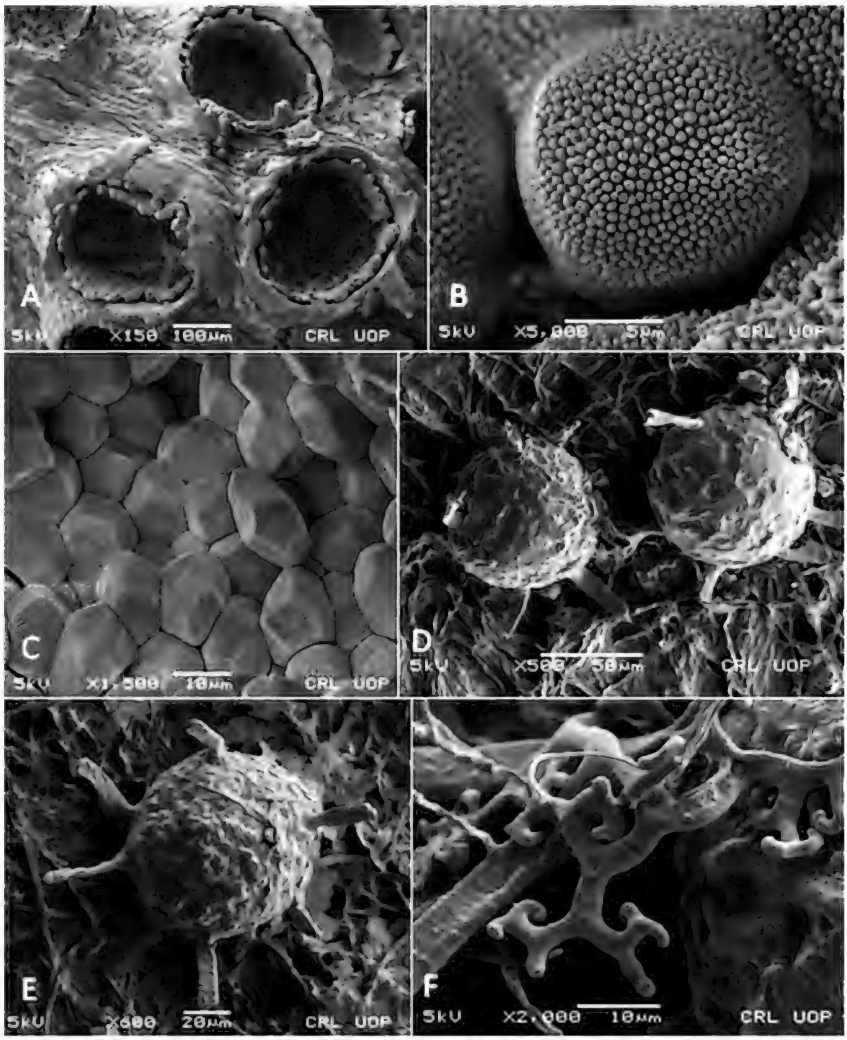

Plate 4. ***Aecidium viburni*** (LAH-AM20002). A. Aecium; B. Aeciospore showing verrucose surface ornamentation; C. Sterile peridial cells. ***Erysiphe viburni*** (LAH-E0001). D, E. Chasmothecium; F. Apical part of dichotomous appendages.

or rarely short-stalked, 6–8 spored; ascospores globose–ellipsoid to ovoid, 16–27 × 11–16 µm, hyaline.

Specimen examined: **PAKISTAN**, **Khyber Pakhtunkhwa**, **Swat district**, Swat, Malam Jabba, at 2250 m asl, on living leaves of *Viburnum grandiflorum*, 11 August 2017, A.N. Khalid & A.R.K. Niazi SS #4 (LAH-E0001).

Comments: *Erysiphe viburni* is a new record for Pakistan. The species has been previously reported on many *Viburnum* species and some members of *Adoxaceae* from Central Asia and China (Cao & al. 2000); India, Japan, Korea (Cho & Shin 2004); and Russia (Far East, Siberia), North America (Canada, USA), and Europe (Braun & Cook 2012).

Erysiphe viburni is distinguished from *E. hedwigii* and *E. miranda* by its larger chasmothecia.

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We sincerely thank Prof. Dr. Uwe Braun (Martin-Luther-Universität, Institut für Biologie, Germany) for his help in the identification of these fungal taxa. The authors also wish to thank Dr. Amy Y. Rossman (Oregon State University, Corvallis) and Dr. Omar Paino Perdomo (Instituto Tecnólogico de Santo Domingo, Dominican Republic) for presubmission reviews of this manuscript. This work was financially supported in part by University of the Punjab, Lahore, Pakistan under a research project entitled 'Taxonomic studies of powdery mildews (*Erysiphales*) on wild plants of Pakistan' for the fiscal year 2015–2016 and in part by Higher Education Commission (HEC), Islamabad, Pakistan under the "Indigenous Ph.D. Fellowship Scheme 5000 Phase II"

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Pluteus umbrosoides and *P. chrysaegis*, new records from China

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Abstract—*Pluteus umbrosoides* and *P. chrysaegis* are reported as new records from China based on morphological observation and molecular evidence. *Pluteus umbrosoides* is characterized by a brown to dark brown squamulose pileus lacking an appendiculate pileus margin, a nearly glabrous stipe, ellipsoid basidiospores, predominantly capitate pleurocystidia, and a trichohymeniderm pileipellis. *Pluteus chrysaegis* is distinguished by its bright yellow pileus, subglobose to globose basidiospores, lageniform to fusoid cheilocystidia with long apical appendages, and abundant fusoid pleurocystidia. Morphologically and phylogenetically both species are members of *P.* section *Hispidoderma*. Morphological descriptions, illustrations, and line drawings of Chinese collections of the two species are provided.

Key words—phylogeny, *Pluteaceae*, taxonomy

Introduction

Pluteus Fr. is the most widespread, saprotrophic, non-ectomycorrhizal, and type genus of the fungal family *Pluteaceae* (Singer 1986, Banerjee & Sundberg 1995, Justo & al. 2011a,b). Approximately 300 species have been reported (Kirk & al. 2008). The traditional subdivision of the genus into three sections (*P.* sect. *Pluteus*, *P.* sect. *Celluloderma* Fayod, and *P.* sect.

Hispidoderma Fayod) is with some rearrangements supported by molecular phylogenetic studies, (Justo & al. 2011a,b; Menolli & al. 2010, 2015a,b). *Pluteus* sect. *Hispidoderma* accommodates those species with non-metuloid pleurocystidia and a pileipellis consisting of elongated, filamentous elements organized as a cutis, hymeniderm, or trichoderm (Menolli & al. 2010; Justo & al. 2011a,b, 2012; Vizzini & Ercole 2011; Lezzi & al. 2014).

Here we present two new records from China, identified by morphological and molecular data: *P. umbrosoides* (originally described from Siberia, Russia; Malysheva & al. 2016) and *P. chrysaegis* (originally described from Sri Lanka; Pegler 1986). The newly recorded species are compared with morphologically similar species of *Pluteus*, and their phylogenetic positions are assessed.

Material & methods

Morphological studies

Fungal specimens of *Pluteus* were collected from China. The examined specimens were deposited in the Fungal Herbarium of the Guangdong Institute of Microbiology, Guangzhou, China (GDGM). Macromorphological descriptions were based on the field notes and documented by photographs.

Micromorphological observations were made from the dried specimens. Water, 5% aqueous KOH (w/v), Congo Red (staining tissues), and Melzer's solution (to detect amyloidity in basidiospores and tissues) were used in rehydration and as mounting media. Basidiospore measurements are annotated with [n/m/p] to denote 'n' basidiospores from 'm' basidiomata of 'p' collections and dimensions given as (a–) b–c(–d), with 'b–c' representing at least 90% of the measured values and extreme values 'a' and 'd' enclosed in parentheses when necessary. Q = length/width ratio per basidiospore, Q_m = average of 'n' basidiospores, SD = standard deviation, with results presented as $Q_m \pm SD$.

Molecular studies

Protocols for genomic DNA extraction, PCR amplification, and sequencing followed Hosen & al. (2013). The ITS1-F/ITS4 (White & al. 1990) primer pair was used to amplify the nuclear ribosomal internal transcribed spacer (ITS) region.

A total of 65 sequences (Fig. 1) representing *Pluteaceae* was retrieved from GenBank based on NCBI blast search results and published analyses (Justo & al. 2011a,b; Menolli & al. 2010, 2015a,b; Pradeep & al. 2012; Malysheva & al. 2016) and combined with five newly generated ITS sequences from Chinese *Pluteus* material. The ITS sequences were aligned in MAFFT v.6.8 (Katoh & al. 2005) with default settings and manually edited in BioEdit v.7.0.9 (Hall 1999). A Maximum Likelihood (ML) analysis was performed using RAxML v.7.2.6 (Stamatakis 2006). As RAxML supports only the GTR model of nucleotide substitution, the GTRGAMMAI model was used for phylogenetic analyses, and statistical support

values were obtained using nonparametric bootstrapping (BS) with 1000 replicates. *Volvariella bombycina* (Schaeff.) Singer was selected as outgroup.

Molecular phylogenetic results

The ITS sequences newly generated from Chinese *Pluteus* specimens were deposited in GenBank (MH059511–MH059514). In the aligned ITS dataset,

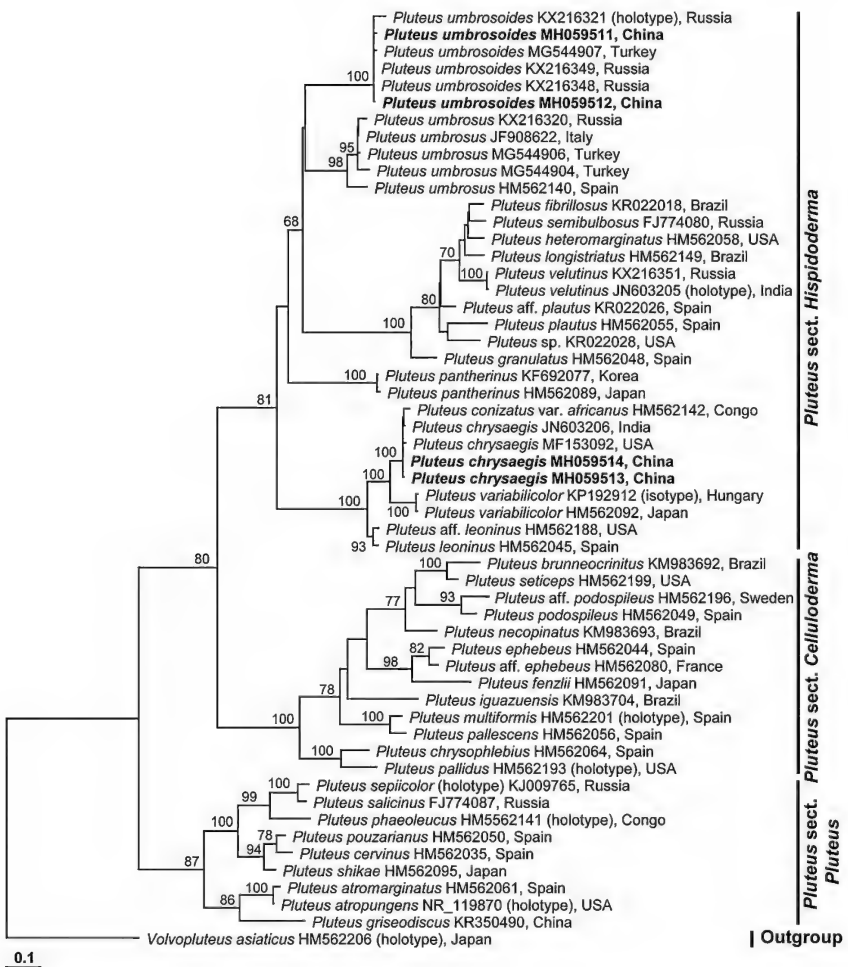
Pluteus umbrosoides KX216321 (holotype), Russia
Pluteus umbrosoides MH059511, China
Pluteus umbrosoides MG544907, Turkey
Pluteus umbrosoides KX216349, Russia
Pluteus umbrosoides KX216348, Russia
Pluteus umbrosoides MH059512, China
Pluteus umbrosus KX216320, Russia
Pluteus umbrosus JF908622, Italy
Pluteus umbrosus MG544906, Turkey
Pluteus umbrosus MG544904, Turkey
Pluteus umbrosus HM562140, Spain
Pluteus fibrillosus KR022018, Brazil
Pluteus semibulbosus FJ774080, Russia
Pluteus heteromarginatus HM562058, USA
Pluteus longistriatus HM562149, Brazil
Pluteus velutinus KX216351, Russia
Pluteus velutinus JN603205 (holotype), India
Pluteus aff. plautus KR022026, Spain
Pluteus plautus HM562055, Spain
Pluteus sp. KR022028, USA
Pluteus granulatus HM562048, Spain
Pluteus pantherinus KF692077, Korea
Pluteus pantherinus HM562089, Japan
Pluteus conizatus var. africanus HM562142, Congo
Pluteus chrysaegis JN603206, India
Pluteus chrysaegis MF153092, USA
Pluteus chrysaegis MH059514, China
Pluteus chrysaegis MH059513, China
Pluteus variabilicolor KP192912 (isotype), Hungary
Pluteus variabilicolor HM562092, Japan
Pluteus aff. leoninus HM562188, USA
Pluteus leoninus HM562045, Spain
Pluteus brunneocrinitus KM983692, Brazil
Pluteus seticeps HM562199, USA
Pluteus aff. podospileus HM562196, Sweden
Pluteus podospileus HM562049, Spain
Pluteus necopinatus KM983693, Brazil
Pluteus ephebeus HM562044, Spain
Pluteus aff. ephebeus HM562080, France
Pluteus fenzlii HM562091, Japan
Pluteus iguazuensis KM983704, Brazil
Pluteus multiformis HM562201 (holotype), Spain
Pluteus pallescens HM562056, Spain
Pluteus chrysophlebius HM562064, Spain
Pluteus pallidus HM562193 (holotype), USA
Pluteus sepiicolor (holotype) KJ009765, Russia
Pluteus salicinus FJ774087, Russia
Pluteus phaeoleucus HM5562141 (holotype), Congo
Pluteus pouzarianus HM562050, Spain
Pluteus cervinus HM562035, Spain
Pluteus shikae HM562095, Japan
Pluteus atromarginatus HM562061, Spain
Pluteus atropungens NR_119870 (holotype), USA
Pluteus griseodiscus KR350490, China
Volvopluteus asiaticus HM562206 (holotype), Japan
Pluteus sect. Hispidoderma
Pluteus sect. Celluloderma
Pluteus sect. Pluteus
Outgroup
0.1

FIG. 1. Phylogenetic relationships of *Pluteus* species and its allied genera inferred from ITS data using ML method. RAxML bootstrap support values ≥50% are indicated on the branches at nodes. Newly generated sequences of *Pluteus* species from China are set in bold font. GenBank accession numbers and country names are provided after the species name. The tree is rooted by *Volvariella bombycina* as the outgroup.

sequences of 55 sampled *Pluteaceae* specimens included 825 nucleotide sites (gaps included) per sample. Our phylogeny supports the Chinese sequences MH059511 and MH059512 as conspecific with *P. umbrosoides* (and closely related to *P. umbrosus* (Pers.) P. Kumm.), and sequences MH059513 and MH059514 as conspecific with *P. chrysaegis* (and closely related to *P. variabilicolor* Babos) (Fig. 1).

Fig. 2. ***Pluteus umbrosoides*** (GDGM 42350): a, b. Natural habit. ***Pluteus chrysaegis*** (GDGM 42287 and GDGM 42376): c. Natural habit (GDGM 42287): d. Natural habit. (GDGM 42376). Scale bars = 10 mm.

Taxonomy

Pluteus umbrosoides E.F. Malysheva, Mycol. Progr. 15: 880 (2016) Figs 2a,b; 3

Basidiomata small to medium-sized. Pileus 35–60 mm broad, hemispherical, convex to applanate with a slightly umbo, depressed in some areas, uneven; brown to dark brown, reddish brown to grayish brown, pale brown towards margin; surface covered by brown to dark brown or blackish brown squamules, more densely at centre; margin uneven, minutely serrate with undulate; pileus context 8 mm thick at centre, white, unchanged when injured. Lamellae 4 mm broad, free, crowded, slightly ventricose, pale pink to pink; lamellulae common at different lengths. Stipe 45–50 × 4–5 mm, cylindrical, slightly thickening towards base, smooth but minutely fibrillose nearly to the stipe base, elsewhere glabrous, pallid white to dull white, slightly

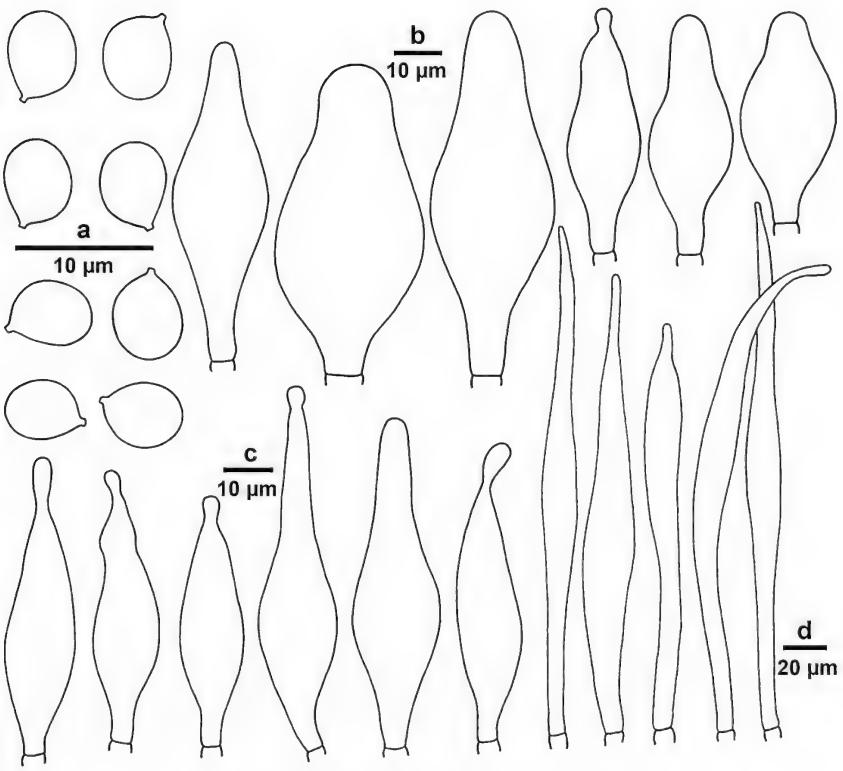

Fig. 3. ***Pluteus umbrosoides*** (GDGM 42350).
a. Basidiospores; b. Cheilocystidia; c. Pleurocystidia; d. Pileipellis elements.

darker at the base, solid to slightly fistulose; context white, unchanged when cut. Odor and taste not recorded.

Basidiospores [40/2/2] 5.5–6.5(–6.8) × (4.5–)5.0–6.0(–6.5) µm [mean length = 6.15, mean width = 5.23, Q = (1.05–)1.10–1.20(–1.23); Q_m = 1.17 ± 0.044), ellipsoid to subglobose, hyaline to pale yellow, thick-walled (0.5 µm thick). Basidia 20–26(–30) × 7–8 µm, 2–4-spored, clavate to narrowly clavate; sterigmata up to 3 µm long. Cheilocystidia 40–75 × 11–31 µm, abundant, fusiform to broadly fusiform, utriform, or broadly lageniform with appendage, sometimes capitulum head present, hyaline, thin-walled. Pleurocystidia 40–80 × 11–18 µm, abundant, fusiform to broadly fusiform, lageniform to broadly lageniform, tapering into apex generally bearing a capitulum head, sometimes also presenting cheilocystidium-like fusiform elements, hyaline, thin-walled.

Pileipellis a trichohymeniderm, composed of narrowly to broadly fusiform elements with tapering or obtuse or papillate apexes, 100–300 × 15–25 µm, with yellow-brown intracellular pigment, thin-walled. Stipitipellis vertically oriented, cylindrical hyphae 5–25 µm wide, pale yellow to yellowish; caulocystidia rare. Clamp connections absent in all tissues.

Specimens examined: **CHINA, Jilin province: Changjiang County**, Bawangling National Forest Park, Changbaishan Mountain, Lushui River, Deer Farm, 11 August 2011, leg. Ming Zhang, Xiao-Lan He, Hao Huang (GDGM29469, GenBank MH059512); 21 August 2012, leg. Jiang Xu (GDGM42350, GenBank MH059511).

Habitat & distribution—Solitary, grows on decayed wood. Recorded from Russia (Siberia) and China (and possibly Turkey, fide GenBank MG544907).

Pluteus chrysaegis (Berk. & Broome) Petch,
Ann. Roy. Bot. Gard. (Peradeniya) 5: 271 (1912) Figs 2c,d; 4

Basidiomata small to medium-sized. Pileus 25–45 mm broad, hemispherical, convex to plano-convex, wrinkled to rugulose in some areas, brilliant golden yellow to chrome yellow, central disc darker and often rugulose, margin finely striate, entire; context 3 mm thick at the pileus center, elsewhere thin, unchanged when cut or injured. Lamellae free, 5 mm broad, not ventricose, white then pale pink, crowded; lamellulae common with different lengths. Stipe 45–65 × 35–47 mm, central, cylindrical, slightly thickening towards the base, glabrous, surface pallid white to pale yellow, usually yellowish to brownish towards the base; basal mycelium whitish to yellowish white; context 2 mm thick, white, unchanged when cut. Taste and odor not recorded.

Basidiospores [40/2/2] 5.0–5.5(–6.2) × 4.5–5.0 µm [mean length = 5.28, mean width = 4.83, Q = (1.02–)1.07–1.15(–1.20); Qm = 1.09 ± 0.051], subglobose, globose to nearly broadly ellipsoid, hyaline to pale yellow, thick-walled (0.5 µm thick). Basidia 25–35 × 6–8 µm, 2–4-spored, clavate to narrowly clavate; sterigmata up to 3 µm long. Cheilocystidia 30–80 × 6–15 µm, abundant, fasciculate, mostly lageniform to fusiform with long appendage toward apex, hyaline, thin to slightly thick-walled. Pleurocystidia 45–75 × 10–20 µm, abundant, fusiform to broadly fusiform, rarely broadly clavate to mucronate, hyaline, thin-walled. Pileipellis an epithelial, intermixed with abundant pileocystidia, clavate cells measuring 15–25 × 6–10 µm, pileocystidia measuring 20–50 × 7–10 µm, similar to cheilocystidia but shorter and narrower, hyaline to pale yellow, thin-walled. Stipitipellis vertically oriented, cylindrical hyphae 4–10 µm wide, pale yellow to yellowish; caulocystidia not found. Clamp connections absent in all tissues.

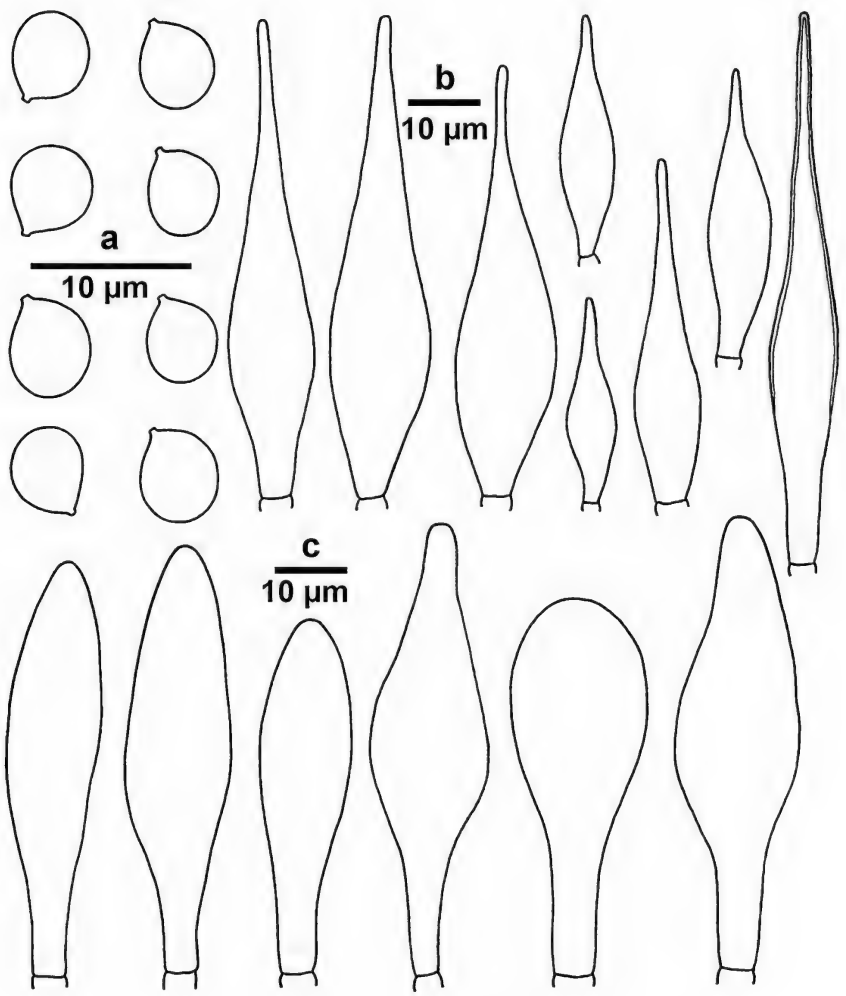

FIG. 4. ***Pluteus chrysaegis*** (GDGM 42287).
a. Basidiospores; b. Cheilocystidia; c. Pleurocystidia.

SPECIMENS EXAMINED: **CHINA, GUANGDONG PROVINCE: Shaoguan City**, Chebaling, Xianrendong National Nature Reserve Forest, 11 July 2013, leg. Jiang Xu, Bin Song, Ting Li and Wei Wang (GDGM42287, GenBank MH059513). **HUNAN PROVINCE: Chenzhou City**, Mangshan National Nature Reserve, 17 August 2014, leg. Xu Jiang, Zhou Shihao (GDGM42376, GenBank MH059514).

Habitat & distribution—Solitary, grows on decayed wood. Recorded from Sri Lanka, India, West Africa, and China (and possibly USA, fide GenBank MF153092).

Discussion

Pluteus umbrosoides, originally described from Russia (Krasnoyarsk, Siberia), is characterized by a brown to dark brown squamulose pileus lacking an appendiculate pileus margin and with squamules often arranged in a vein-like pattern, nearly smooth stipe, ellipsoid basidiospores, predominantly capitate pleurocystidia, and a trichohymeniderm pileipellis (Malysheva & al. 2016).

Our molecular phylogenetic analysis places the Chinese collections (GDGM42350 and GDGM29469) in *P.* sect. *Hispidoderma* and nested in the same lineage as the type material of *P. umbrosoides* (Fig. 1). Based on 620 nucleotides from the ITS sequence, genetic distances among the Russian collections (including type material), Turkish collection, and Chinese collections range from 0.32–0.82%, suggesting that all collections represent one species with intraspecific variation rather than being different species.

Pluteus umbrosoides is morphologically similar to *P. umbrosus* and *P. granularis* Peck. However, both of those species are distinguished from *P. umbrosoides* by their brown edged lamellae, a pileus margin that is distinctly appendiculate or covered with over-hanging floccules, non-smooth stipe surface, a stipe surface abundantly covered by caulocystidia, and longer pileipellis elements (Malysheva & al. 2016). Phylogenetically, *P. granularis* and *P. umbrosus* cluster together with strong BS support value and are separated from *P. umbrosoides* (Fig. 1). Geographically, *P. umbrosus* is distributed in Eurasia (Spain, Italy, Russia, and Turkey) and *P. granularis* is recorded only in USA, while *P. umbrosoides* is found in Russia (Siberia) and now China.

Pluteus chrysaegis, originally described from Sri Lanka, is characterized by a bright yellow pileus, subglobose to globose basidiospores, lageniform to fusoid cheilocystidia with long appendages, abundant fusiform pleurocystidia, and a pileipellis composed of clavate cells intermixed with fusiform pileocystidia (Pegler 1986, Pradeep & Vrinda 2006, Pradeep & al. 2012, Desjardin & Perry 2018). Initially, *P. chrysaegis* was placed in *P.* sect. *Celluloderma* based on the greater number of fusiform elements in the pileipellis (Pegler 1986, Pradeep & Vrinda 2006), but subsequent

molecular studies (Pradeep & al. 2012, Desjardin & Perry 2018, this paper) place it in *P.* sect. *Hispidoderma*.

Pluteus chrysaegis was known only from the type locality (Pegler 1986), until it was found in India (Pradeep & Vrinda 2006, Pradeep & al. 2012), West Africa (Desjardin & Perry 2018), and now China. One sequence (GenBank MF153092) from USA also clusters with *P. chrysaegis* (Fig. 1), suggesting that this species has a wide geographic distribution ranging from South Asia, East Asia, West and Equatorial Africa, and North America. *Pluteus conizatus* var. *africanus* E. Horak, originally described from Equatorial Africa (Horak 1977), nests in the same lineage and is considered to represent a synonym of *P. chrysaegis* (Pradeep & al. 2012, Desjardin & Perry 2018).

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Five new *Escovopsis* species from Argentina

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Abstract—*Escovopsis atlas, E. catenulata, E. longivesica, E. primorosea,* and *E. pseudoweberi* spp. nov. are described from fungal gardens of higher attine ants in Argentina. All these species possess clear diagnostic features: *E. atlas* characterized by subglobose vesicles; *E. catenulata* by the presence of claviform vesicles forming short chains; *E. longivesica* by longer vesicles than the rest of the species; *E. primorosea* by pink coloration of colony in mCYA medium; and *E. pseudoweberi* by the smallest conidia known in the genus.

Key words—*Acromyrmex*, ant nest parasite, *Atta*, *Hypocreales*, taxonomy.

Introduction

Escovopsis J.J. Muchovej & Della Lucia (*Hypocreales*) is a symbiotic genus of mycoparasites of attine ant nests (Currie & al. 2003). Muchovej & Della Lucia (1990) proposed *Escovopsis* as a substitute for the invalid genus "*Phialocladus*" Kreisel, which lacked a designated type (Kreisel 1972). The authors designated *Escovopsis weberi,* isolated from a fungal garden of *Atta* sp. in Minas Gerais, Brazil, as the type species of the genus. Seifert & al. (1995) described a second

species, *E. aspergilloides* Seifert & al., isolated from a nest of *Trachymyrmex* from Trinidad; they clarified the ontogeny in the genus and confirmed that the conidiogenous cells of *Escovopsis* species were phialidic, in agreement with Kreisel's (1972) original description of "*Phialocladus*".

Augustin & al. (2013) published three new species from nests of *Acromyrmex* in Minais Gerais, Brazil: *Escovopsis lentecrescens* H.C. Evans & J.O. Augustin, *E. microspora* H.C. Evans & J.O. Augustin, and *E. moelleri* H.C. Evans & J.O. Augustin; they also described *Escovopsioides* H.C. Evans & J.O. Augustin as a morphologically and molecularly distinct genus also associated with attine ant nests. Augustin & al. (2013) laid the groundwork for the current molecular taxonomy of *Escovopsis,* including new species and comparing them with previously described morphotypes from ecological and evolutionary research (Currie & al. 2003, Taerum & al. 2007, Gerardo & al. 2006).

Two additional *Escovopsis* species have been published: *E. kreiselii* L.A. Meirelles & al. from a nest of *Mycetophylax morschi* in Santa Catarina, Brazil; and *E. trichodermoides* M. Cabello & al. from a nest of *Mycocepurus goeldii* in São Paulo, Brazil (Masiulionis & al. 2015, Meirelles & al. 2015).

Additionally, Gerardo & al. (2006) isolated and characterized several *Escovopsis* strains from *Apterostigma* ants in Panama, Costa Rica, and Argentina, grouping the strains by coloration of mass conidia (brown, yellow, white, and pink), contrasting with the exclusively brown conidia of all described species of *Escovopsis*. No further details on the taxonomy of these morphotypes were mentioned, making it impossible to assign them formally to any *Escovopsis* species; but informally, and based on phylogenetic information, these morphotypes were considered members of *Escovopsis* (Gerardo & al. 2006). Brown conidial morphotypes were associated with the ant genera *Trachymyrmex*, *Apterostigma, Atta,* and *Acromyrmex;* white conidial morphotypes with *Apterostigma* and *Cyphomyrmex;* yellow conidial morphotypes with *Apterostigma;* and pink conidial isolates with *Cyphomyrmex* and *Apterostigma* (Gerardo & al. 2006).

The present work aims to describe, both morphologically and molecularly, new species of *Escovopsis* found in nests of *Acromyrmex* and *Atta* during a biodiversity study of the genus *Escovopsis* from different phytogeographical regions of Argentina.

Materials & methods

Fungal Isolates—Between June 2006 and November 2012, we sampled nests of leaf-cutting ants from different Argentinean provinces to isolate *Escovopsis* strains.

Twenty-six isolates of *Escovopsis* were obtained from garden material from different sites and different ant nests. Fungal isolations were made in the field and were conducted by collecting small individual pieces of fungus garden material (~2 mm^3) and placing them on PDA (Potato dextrose agar) plates with Penicillin–G (100 U/ml) with the pH of culture media adjusted to pH 7. Twenty samples from each nest were taken in situ from different areas of the cultivar garden. All isolates were taken to the laboratory and incubated 7–10 days at 25 °C. After incubation, morphological distinct isolates were recovered and subcultured in additional plates in order to obtain pure cultures. *Escovopsis* isolates were maintained as live cultures in PDA until conidia and mycelia were observed and then preserved in glycerol (20% v/v) at –80 °C. Holotypes were deposited as dried collections in the Herbarium, Universidad de Buenos Aires, Argentina (BAFC). Ex-holotype and additional strains examined were deposited in the culture collection of the Quilmes National University, Bernal, Buenos Aires, Argentina (UNQ).

Morphological analysis—All isolates were identified morphologically using macro- and microscopic characters. Isolates were grown on PDA and on modified Czapek yeast agar (mCYA) composed of 0.03 M KNO_3, 5.74×10^{-3} M K_2HPO_4, 6.7×10^{-3} M KCl, 2.02×10^{-3} M $MgSO_4 + 7H_2O$, 3.7×10^{-4} M $FeSO_4 + 7H_2O$, 8.7×10^{-6} M $ZnSO_4+7H_20$, 2×10^{-5} M $CuSO_4+5H_2O$, 5g/L Yeast Extract (DIFCO), 30g/L dextrose, 20g/L agar. After the cultures were incubated at 25 °C, 80% RH, in darkness for 7 d, the colony diameter was measured using the software ImageJ V1.47. The coloration of the colony was coded according to Maerz & Paul (1930). For microscopic studies, fungal material was mounted in water and observed under a Nikon Eclipse E200 compound microscope; to improve visualization, staining with methylene blue, Congo red, and phloxine was used. The following microscopic characters were observed: shape, size, and ornamentation of conidia, shape and size of conidiogenous cell and vesicles, and diameter of vegetative hyphae. Each character was measured at least twenty times per isolate. To improve measurement precision, conidial size was measured using the software Micrometrics TM SE Premium 2.8234.

All isolate measurements were compared to those of the seven described species of *Escovopsis* in the literature. Strains that did not agree with any described species were clustered into morphotypes, which were also described. For *E. weberi* and *E. aspergilloides* we followed the original descriptions of Muchovej & Della Lucia (1990) and Seifert & al. (1995), as well as the commentaries of Augustin & al. (2013). Although previous *Escovopsis* studies refer to 'spores' (Gerardo & al. 2006, Masiulionis & al. 2015, Meirelles & al. 2015), we use the word 'conidia' as the correct terminology for the asexual reproductive structures.

DNA extraction—Total genomic DNA was extracted by a modification of the method published by Augustin & al. (2013). Isolates were cultured in PDA for seven days at 25 °C and 80 % RH in darkness. Mycelia (50 mg) were placed in a mortar at –80 °C with 500 µL of CTAB buffer (2% cetyltrimethyl ammonium bromide, 1.4 M NaCl at pH 8.0, 20 mM EDTA at pH 8.0, 2% Tris–Cl at pH 8.0 and 1% β–

mercaptoethanol). Using a ceramic pestle, fungal material was ground and later placed in a 1.5 ml microtube. Material was centrifuged for 30 s at 14,500 rcf and then 500 µL of chloroform were added, mixed and centrifuged 15 min at 14,500 rcf. Finally, the aqueous phase was transferred into a new microtube and one volume of cold isopropanol (−20 °C) was added. Samples were precipitated overnight at −20 °C and then centrifuged 15 min at 14,500 rcf to recover DNA. DNA pellets were air dried and resuspended in 50 µL of TE buffer (10 mM Tris–HCl pH 7.5; 0.1 mM EDTA). DNA concentration was determined by Nanodrop 2000 (Thermo Scientific), and integrity was examined in 0.8% agarose gels.

PCR amplification and sequencing—Two gene regions were analyzed: the nuclear D1/D2 domains of 28S rDNA gene and the TEF1 gene encoding translation elongation factor 1α (EF–1α). The 28S rDNA PCR was conducted with the primers CLA–F (5′–GCATATCAATAAGCGGAGGA–3′) and CLA–R (5′–GACTCCTTGGTCCGTGTTTCA–3′) (Currie & al. 2003); and the EF–1α using the primers EF1–5rl (5′–GTGATACCACGCTCACGCTC–3′) and EF1–3F (5′–CACGTCGACTCCGGCAAGTC–3′) (Gerardo & al. 2004). Amplification of the 28S rDNA fragment was carried out in 50 µL with 1× PBL Taq Buffer, 3 mM $MgCl_2$; 0.2 mM of each dNTP; 1 U of Pegasus Taq; 0.4 µM of each primer and 75–100 ng DNA and purified H_2O to complete volume. PCR conditions were: 2 min at 95 °C, 40 cycles with 30 s at 95 °C, 60 s at 52 °C, and 90 s at 72 °C; and a final step with 5 min at 72 °C. The amplification of TEF1 amplification was carried out in 50 µL with 1× PBL Taq Buffer, 3 mM of $MgCl_2$; 0.2mM from each dNTP; 1 U of Taq (Pegasus model); 0.4 µM of each primer (PBL company); 25–100 ng of DNA, and purified H_2O to complete volume. PCR conditions were: 2 min at 95 °C, 40 cycles with 30 s at 95 °C, 60 s at 52 °C and 90 s at 72 °C; and finally, 5 min at 72 °C. All PCR reactions were done in a Veriti 96-well thermal cycler. PCR products were purified and sequenced by Macrogen Corporation.

Phylogenetic analyses—All sequences generated in this study and sequences available in GenBank were aligned using ClustalW (Thompson & al. 1994) and manually edited using MEGA 5 (Tamura & al. 2013). Additionally, poorly aligned portions and divergent positions (ambiguous positions) were deleted using CSIC-UPF Gblocks with less stringent options. Phylogenies were reconstructed using three approaches: maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI). For the analyses, complete deletion of gaps for 28S rDNA and partial deletion (80%) for TEF1 sequences were used.

For ML analysis, the DNA sequence evolution model was established based on the Akaike information criterion (AIC) implemented in MEGA 5. The Tamura–Nei model was selected for 28S rDNA with a Γ distribution to model evolutionary rate differences among sites (2 categories (+G, parameter = 0.3419)). For TEF1, the Kimura 2–parameter model with a Γ distribution to model evolutionary rate differences among sites (2 categories (+G, parameter = 1.4362)) was used. The initial tree for the heuristic search was obtained by applying the neighbour–joining method to a matrix of pairwise

distances estimated by the maximum composite likelihood (MCL) approach. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. Heuristic ML bootstrap analysis consisted of 1000 pseudoreplicates.

We ran MP analysis in MEGA 5 using heuristic searches with 300 random-addition sequence replicates. Tree-bisection reconnection (TBR) branch swapping was also performed. Heuristic MP bootstrap analysis consisted of 1000 pseudoreplicates (TBR branch swapping).

The BI analysis was performed in MrBayes v. 3.1 (Ronquist & Huelsenbeck 2003). We calculated posterior probabilities by a Metropolis–coupled Markov chain Monte Carlo analysis until the runs converged with a split frequency of 0.01. We employed the General Time Reversible model with a proportion of sites invariant and gamma–distributed rates (GTR+I+G). We used a sample frequency of 1000 independent analyses, with ten million generations each. The first 25% generations were discarded as "burn–in". The strict consensus trees and posterior probabilities were calculated from 15,000 trees.

The Figtree V1.4.2 program was used to obtain the final tree and to analyse the Bayesian probability.

Phylogenetic results

Among the 26 isolates studied, five matched the description of Muchovej & Della Lucia (1990) for *E. weberi*. In addition, five morphotypes presented marked differences from all previously described species. Morphological and phylogenetic analyses support these morphotypes as independent species. Among the most important characteristics, we can highlight the morphology and size of vesicles, conidial shape and size, and colony coloration in mCYA. Five new *Escovopsis* species are proposed: *E. atlas, E. catenulata, E. longivesica, E. primorosea,* and, *E. pseudoweberi.*

The matrix for the 28S rDNA gene comprised 38 sequences and 484 characters including 87 conserved sites, 397 variable sites, and 168 parsimony informative characters. On the other hand, the data matrix for the TEF1 gene comprised 53 sequences and 134 characters including 3 conserved sites, 134 variable sites, and 127 parsimony informative characters. All sequences were deposited in GenBank under accession numbers KU298275–KU298308. Alignments were deposited in TreeBASE under accession number 18631.

We obtained robust phylogenies for both markers, 28S rDNA and TEF1, showing no significant differences in the position of the main clades including *Escovopsis* reference sequences from GenBank and the sequences obtained in this study. Maximum likelihood trees are presented in Fig. 1 for 28S rDNA and in Fig. 2 for TEF1. Multilocus phylogenies were not possible using both markers because several isolates with TEF1 sequences (yellow, white, and pink

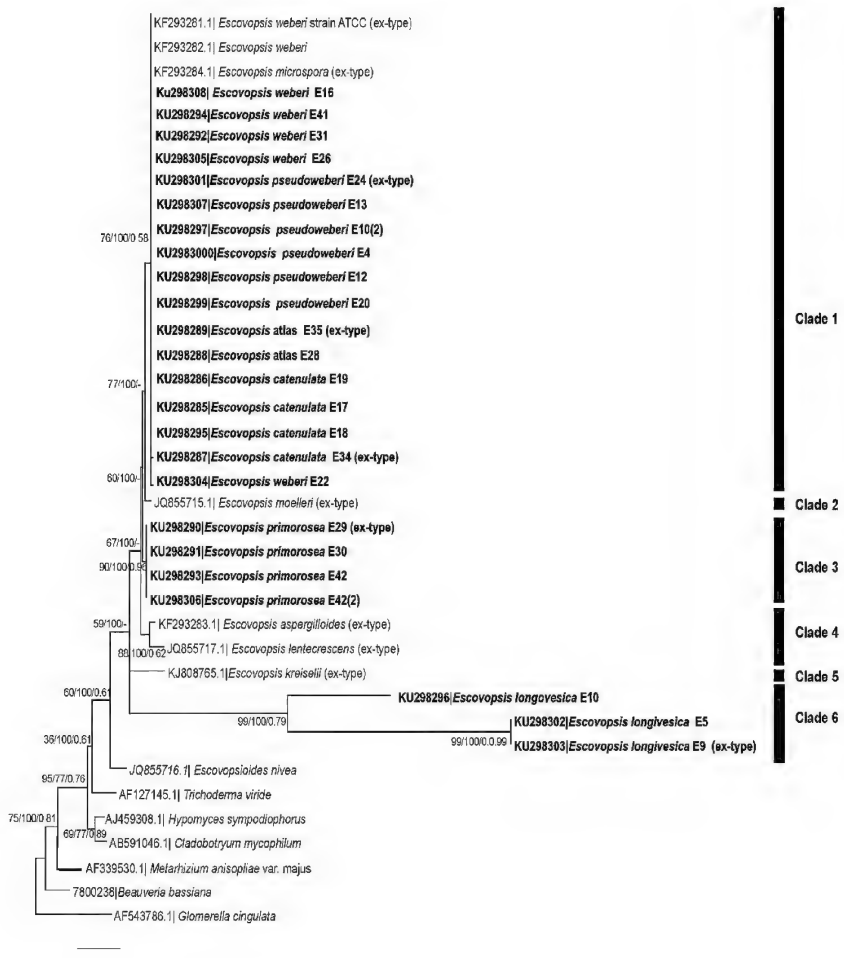

FIG. 1: Phylogeny of *Escovopsis* illustrating species relationships inferred from joint ML analysis of 28S rDNA sequence analysis. The series of three values at nodes correspond to ML and MP bootstrap values > 75%, and, BI posterior probabilities > 0.90.

conidiated clades and *E. trichodermoides*) were not available in GenBank for 28S rDNA.

Phylogenetic analyses of 28S rDNA gene (FIG. 1) support *Escovopsis* as a monophyletic group, where six clades were defined. Clade 1 included the type species of the genus, *E. weberi*, and closely related isolates, with varying

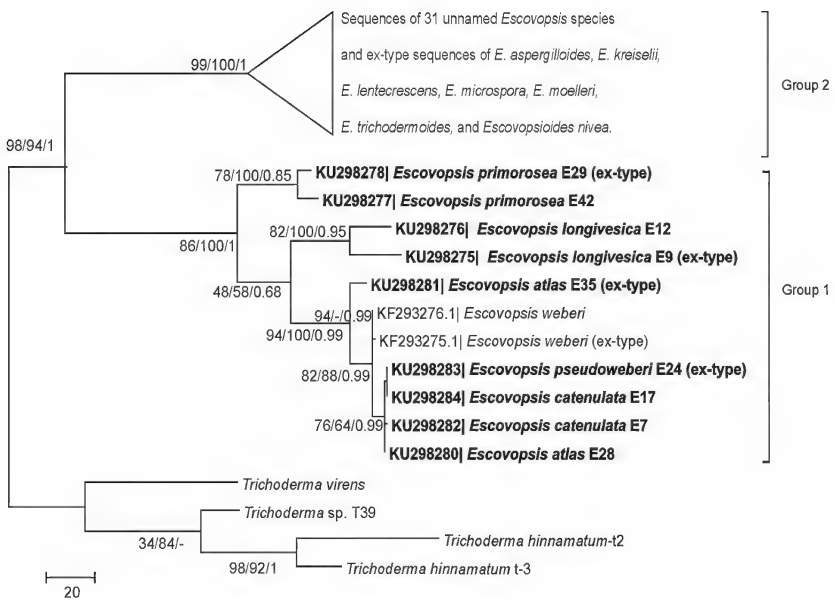

FIG. 2: Phylogeny of *Escovopsis* illustrating species relationships inferred from joint ML analysis of TEF1gene analysis. The series of three values above internal branches correspond to ML and MP bootstrap values > 75%, and BI posterior probabilities > 0.90.

levels of support (ML = 100%, MP = 76%, BI = 0.58). Clade 1 is composed of *E. weberi* (KF293281, KF293282), *E. microspora* (KF293284), *E. weberi* isolates from Argentina (E16, E41, E31, E22, E26), and representatives of three new species: *E. pseudoweberi* (E4, E13, E10(2), E12, E20), *E. atlas* (E28, E35), and *E. catenulata* (E17, E18, E19, E34). The 28S rDNA marker failed to separate these species into monophyletic groups presenting a single polytomy. Clade 2 is composed of a single reference sequence for *E. moelleri* (JQ855715) with high moderate support (ML = 77%, MP = 100%). Clade 3 grouped isolates E29, E30, and E42 of the new species *E. primorosea* in a well-supported monophyletic group (ML = 90%, MP = 100%, BI = 0.96). Clade 4 is formed by the reference sequences of *E. aspergilloides* (KF293283) and *E. lentecrescens* (JQ855717) with good support (ML = 88%, MP = 100%) but low posterior probability (BI = 0.62). Clade 5 is represented by a single lineage containing *E. kreiselii* (K808765); its relationship to other *Escovopsis* species is unresolved. Finally,

the basal Clade 6 appears as a well-supported group composed of isolates E5, E9, and E10 of the new species *E. longivesica* (ML = 99%, MP = 100%, BI = 0.79). In this phylogeny, the reference sequence of *Escovopsioides* (JQ8557161) did not group with any *Escovopsis* sequences.

In the phylogenetic analyses of the TEF1 gene (Fig. 2), the highly supported clade (ML = 86%, MP = 100%, BI = 1) of brown conidiated species, Group 1, contains *E. weberi* and all of our new *Escovopsis* species and several unidentified isolates from previous studies. Within Group 1, there were well-supported clades for *E. longivesica* (ML = 82%, MP = 100%, BI = 0.95) and *E. primorosea* (ML = 78%, MP = 100%, BI = 0.85), separating them from the other three new *Escovopsis* species from Argentina and from *E. weberi* (Fig. 2). *Escovopsis weberi* (the generic type), *E. atlas*, *E. pseudoweberi*, and *E. catenulata* were associated in a monophyletic group (ML = 94%, MP = 100%, BI = 0.99) with undetermined relationships. Group 2 is formed by a well-supported clade (ML = 99%, MP = 100%, BI = 1) containing the reference sequence (ex-type) of *E. aspergilloides* (AY172632), *E. kreiselii* (KJ808766), *E. lentecrescens* (JQ855714), *E. microspora* (KJ935030), *E. moelleri* (JQ855712), *E. trichodermoides* (KF033128), *Escovopsioides nivea* (JQ855713) and several sequences from unidentified *Escovopsis*.

Taxonomy

Escovopsis primorosea Marfetán, A.I. Romero & Cafaro, **sp. nov.** Fig 3, 8 d

MycoBank MB 816915

Differs from other *Escovopsis* species by its pink colony coloration on mCYA medium, and its simultaneous production of cylindrical, claviform (solitary and catenulate), and subglobose vesicles.

Type—Argentina, Tucumán, Parque Nacional Campo de los Alisos, (27°13'51"S 65°54'46"W), 1500 m asl, fungal garden of *Acromyrmex aspersus* F. Smith, Nov 2012, J.A Marfetán E29 (**Holotype**, BAFC-H 52761; culture ex type culture, UNQ E29; GenBank KU298290, KU298278).

Etymology—In reference to the coloration of the colony and conidia on mCYA, which is pink initially, but later becomes brown.

Colonies fast growing, colony diameter 5.28–7.04 cm after 7 days on PDA. On mCYA, colony white turning pale pink after four days, then light brown at five days. In PDA, colony white becoming light brown after seven days. Hyphae hyaline, smooth, septate, with Woronin bodies highly visible, 6.2–24.7 µm diam. Conidiophores hyaline, orthogonal branched, cylindrical. Vesicles cylindrical to claviform, with a rounded apex, 15–62.5 (–81.25) × 6.25–26.25 µm and occasionally catenulate, generally with two vesicles

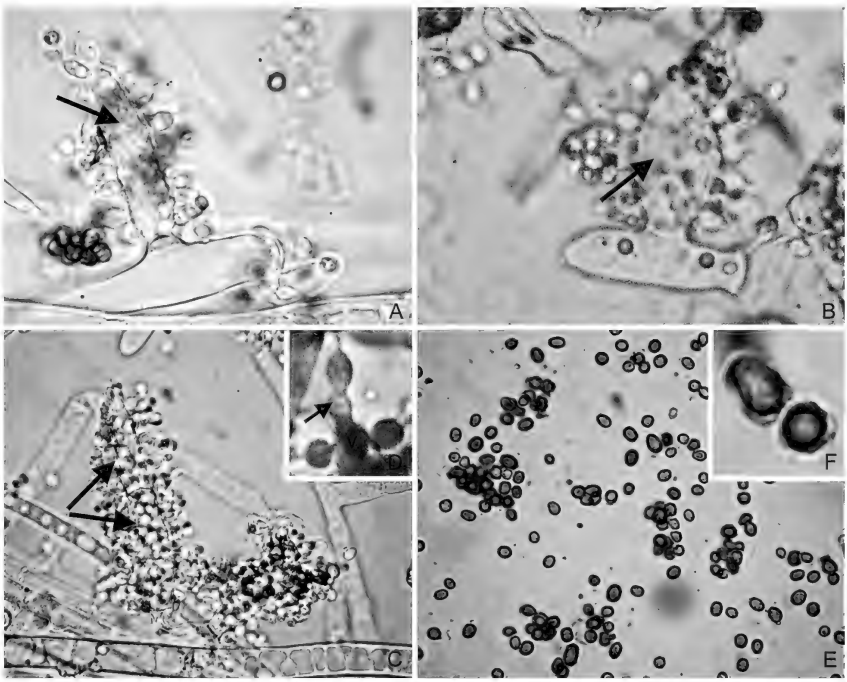

FIG. 3: *Escovopsis primorosea* (holotype, BAFC-H 52761). A. Conidiophore (arrow); B. Vesicle (arrow); C. Catenulate vesicles (arrow); D. Support cell separate (arrow) from the vesicle by septum (v = vesicle); E. Conidia; F. Conidia with cap-like structure.

(12.5–62.5 × 6.25–21.25 µm), occasionally ovoid to subglobose, simple vesicles, 15–27.5 × 12.5–26.25 µm and rarely catenulate, generally with two vesicles. CONIDIOGENOUS CELLS phialidic, with rounded base, elongated neck and inconspicuous collarette, discrete, hyaline, 2.5–12.5 × 1.25–3.74 µm, at the apex of the vesicle conidiogenous cells over a hyaline and octagonal supporting cell (3.1–5 × 5–6.25 µm). CONIDIA with basipetal development, catenulate, ovoid to ellipsoid, 2.5–5 × 2.5–6.25 µm, hyaline becoming brown later, cap–like structure present.

ADDITIONAL SPECIMENS EXAMINED—**ARGENTINA**, **TUCUMÁN**, Parque Nacional Campo de los Alisos, 27°13′51″S 65°54′47″W, 1500 m asl, fungal garden of *Acromyrmex aspersus*, Nov 2012, J.A. Marfetán E30 (UNQ E30; GenBank KU298291, KU298279); J.A. Marfetán E42 (UNQ E42; GenBank KU298293, 298277); J.A. Marfetán E42(2) (UNQ E42(2); GenBank KU298306).

COMMENTARY—*Escovopsis primorosea* can be easily distinguished from the other species by its pink coloration in mCYA and by the simultaneous presence

of cylindrical, claviform (solitary and in chains), and subglobose vesicles. This species can also be diagnosed by 28S rDNA and TEF1 genes.

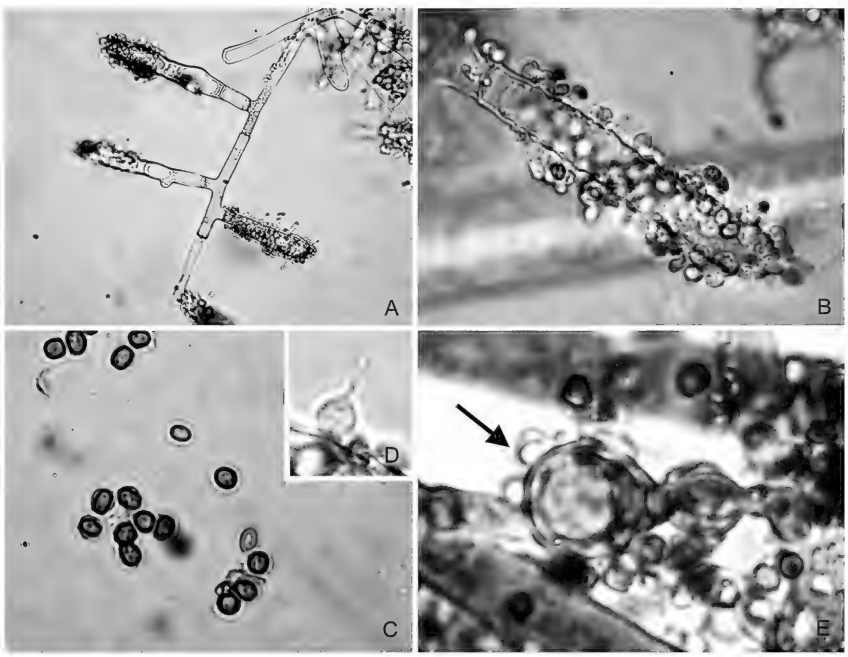

Fig. 4: *Escovopsis longivesica* (holotype, BAFC-H 52762). A, B. Vesicles; C. Conidia; D. Conidiogenous cell; E. Chlamydospore.

Escovopsis longivesica Marfetán, A.I. Romero & Cafaro, **sp. nov.** Figs 4, 8a

MycoBank MB816916

Differs from other *Escovopsis* species by its longer vesicles.

Type—Argentina, La Pampa, Parque Nacional Lihuel Calel, 38°00′06″S 65°35′42″W, 325 m asl, fungal garden of *Acromyrmex striatus* Roger, Oct 2012, J.A. Marfetán E9 (**Holotype**, BAFC-H 52762; ex-type culture, UNQ E9; GenBank KU298303, KU298275).

Etymology—In reference to the conidiophores with longer vesicles.

Colonies in PDA, colony diameter 4.3–7.8 cm after 7 days, initially white in color becoming brown after seven days, abundant aerial mycelium. On mCYA with similar morphology as above. Hyphae hyaline, septate, 3.7–20 µm diam. Conidiophores hyaline growing from the aerial mycelium, with cylindrical, orthogonal branched. Vesicles mainly cylindrical, occasionally

claviform, 6.2–20 × 18.7–62.5 (–75) µm, with a rounded apex, solitary and terminal. Conidiogenous cells phialidic, with rounded base and elongated neck and inconspicuous collarette, 2.5–5 × 2.5–15 µm. Conidia catenulate, with basipetal development, subglobose to ovoid (occasionally ellipsoid), 1.17–2.87 × 1.7–5 µm, hyaline becoming brown later, thick–walled, cap–like structure present. Chlamydospores occasionally present, globose to subglobose, 11.5–12.2 × 11.1–12.7 µm.

Additional specimens examined— **ARGENTINA**, **Buenos Aires**, Mercedes, 34°39′44″S 59°27′07″W, 38 m asl, fungal garden of *Acromyrmex lundii* Guérin-Méneville, Sep 2008, J.A. Marfetán E10 (UNQ E10; GenBank KU298296); Gonnet, 34°52′46″S 58°00′41″W, 3 m asl, fungal garden of *Acromyrmex lundii*, May 2009, J.A. Marfetán E5 (UNQ E5; GenBank KU298302). **La Pampa**, Toay, 36°32′42″S 64°03′01″W, 174 m asl, fungal garden of *Acromyrmex striatus*, Oct 2011, J.A. Marfetán E12 (UNQ E12; GenBank KU298276).

Commentary—*Escovopsis longivesica* is morphologically easily distinguished from other described species by the greater length of its vesicles. This species is separated from *E. aspergilloides, E. lentecrescens,* and *E. primorosea* by the absence of globose to subglobose vesicles. In addition, it is distinguished from *E. weberi, E. moelleri,* and *E. microspora* by the vesicle length (longer in *E. longivesica*) and conidial size (bigger in *E. longivesica*). This species can also be distinguished from the other species using 28S rDNA and TEF1 sequences.

Escovopsis pseudoweberi Marfetán, A.I. Romero & Cafaro, **sp. nov.** Figs 5, 8f

MycoBank MB 816917

Differs from *Escovopsis weberi* and *E. microspora* by its smaller conidia.

Type— Argentina, Tucumán, Parque Nacional Campo de los Alisos, 27°13′50″S 65°55′51″W, 1500 m asl, fungal garden of *Acromyrmex aspersus*, Nov 2012, J.A. Marfetán E24 (**Holotype**, BAFC-H 52763; ex-type culture, UNQ E24; GenBank KU298301, KU298283).

Etymology—Fake *E. weberi,* due to close similarities with *E. weberi.*

Colonies in PDA, colony diameter 5.2–7.95 cm after 7 days, initially white in color becoming brown after five days. Hyphae hyaline, smooth, septate, 3.75–17.5 µm diam, Woronin bodies conspicuous. Conidiophores terminal, hyaline, with cylindrical cells and orthogonal ramifications, bearing numerous vesicles covered by conidiogenous cells. Vesicles claviform, occasionally cylindrical, with a rounded apex 6.2–21.2 × 20–66.2 µm, developing laterally and in the apex of the conidiophores. Conidiogenous cells with rounded base and elongated neck and inconspicuous collarette, phialidic (2.5–5 ×

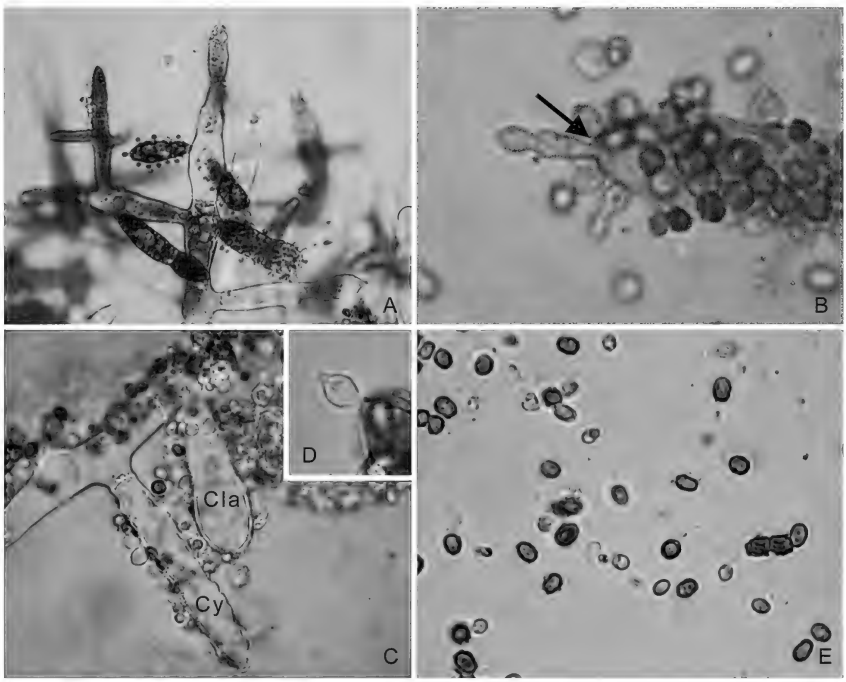

FIG. 5: *Escovopsis pseudoweberi* (holotype, BAFC-H 52763). A. Conidiophores; B Conidiogenous cell with a supporting cell (arrow); C. Cylindrical (Cy) and claviform (Cla) vesicles; D. Conidiogenous cell; E- Conidia.

3.7–12.5 µm), occasionally in the vesicle apex subulate or lageniform (14.5 × 3.75–5 µm) over a refinement at the apex of the vesicle. CONIDIA catenulate, with basipetal development, subglobose to ovoid, sometimes ellipsoid, 0.9–2.7 × 1.2–4.2 µm, hyaline becoming brown with days, rough–walled, cap–like structure occasionally present.

ADDITIONAL SPECIMENS EXAMINED—**ARGENTINA**, **CORRIENTES**, Mercedes, 29°11′50″S 58°02′28″W, 98 m asl, fungal garden of *Acromyrmex lundii*, Mar 2008, J.A. Marfetán E10(2) (UNQ E10(2); GenBank KU298297); J.A. Marfetán E13 (UNQ E13; GenBank KU298307); Mercedes, 29°11′57″S 58°02′18″W, 98 m asl, fungal garden of *Acromyrmex heyeri* Forel, Mar 2008, J.A. Marfetán E12 (UNQ E12; GenBank KU298298). **SANTA FE**, San Cristobal, 30°22′56″S 61°14′16″W, 67 m asl, fungal garden of *Acromyrmex lobicornis* Emery, Aug 2008, J.A. Marfetán E20 (UNQ E20; GenBank KU298299). **BUENOS AIRES**, Gonnet, Estación de Cría de Animales Silvestres (ECAS), 34°50′44′S 58°07′05″W, 3 m asl, fungal garden of *Acromyrmex lundii*, Mar 2009, J.A. Marfetán E4 (UNQ E4; GenBank KU298300).

Commentary—*Escovopsis pseudoweberi* is very similar to *E. weberi* and to *E. microspora* but is distinguished from them by its smaller conidia. Conidial size ranges are 2.5–3.5 × 3.5–4.5 µm for *E. weberi* and (1.5–)2–3 × (2–)2.5–3 µm for *microspora* with *E. pseudoweberi* presenting the smallest range. *Escovopsis pseudoweberi* can also be distinguished from other species using 28S rDNA and TEF1 sequences.

Escovopsis atlas Marfetán, A.I. Romero & Cafaro, **sp. nov.** Figs 6, 8c

MycoBank MB 816918

Differs from other *Escovopsis* species by its subglobose vesicles supported by an octagonal cell.

Type—Argentina, Tucumán, Parque Nacional Campo de los Alisos, 27°13′51″S 65°54′47″W, 1500 m asl, fungal garden of *Acromyrmex aspersus*, Nov 2012, J.A. Marfetán E35 (**Holotype**, BAFC-H 52764; ex-type culture, UNQ E35; GenBank KU298289, KU298281).

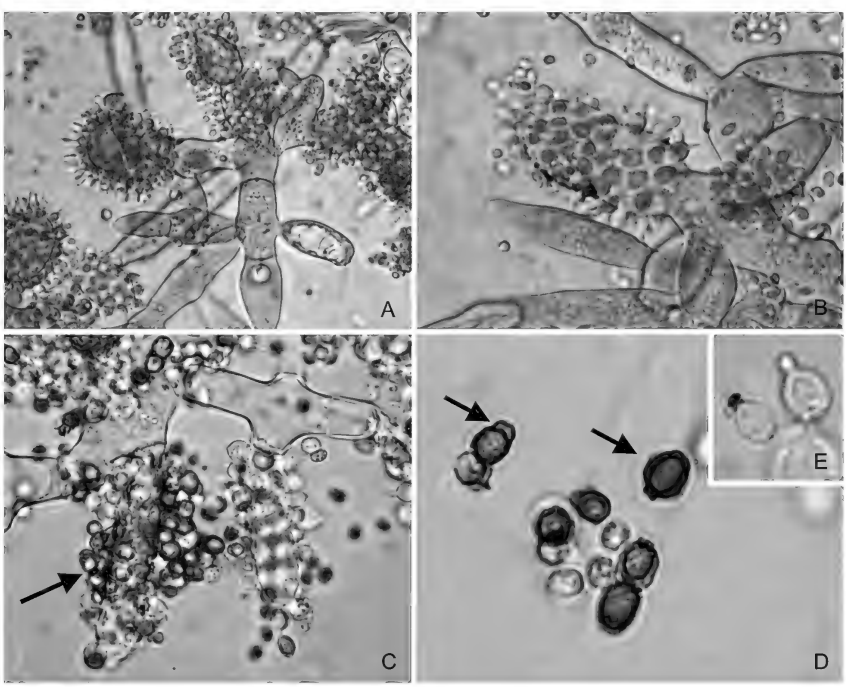

Fig. 6: *Escovopsis atlas* (holotype, BAFC-H 52764). A. Conidiophore (arrow); B. Catenulate subglobose vesicles separated (arrow); C. Cylindrical vesicles; D. Conidia with cap-like structure (arrow); E. Conidiogenous cells.

Etymology—Greek, *Atlas*, a divinity who bears the globe on his shoulders. This is a reference to the subglobose vesicles (= globe) supported by an octagonal cell (= shoulders).

Colonies in PDA, colony diameter 5.5–7.9 cm at day 7, initially white in color becoming brown after 5 days, abundant aerial mycelium. Hyphae hyaline, septate, 6.25–5 µm diam, with Woronin bodies highly visible. Conidiophores, hyaline growing from the aerial mycelium, cylindrical cells and orthogonal branched. Vesicles subglobose (8.8–27.5 × 16.2–31.2 µm) and claviform (18.7–52.5 × 7.5–30 µm), occasionally subglobose catenulate (18.7–23.7 × 20–21.7 µm), supported by octagonal cells (19.3–30 × 23.3–40.5 µm). Conidiogenous cells phialidic, with rounded base, elongated neck and inconspicuous collarette, 2.5–5 × 3.7–8.7 µm. Conidia catenulate, with basipetal development, cylindrical to ellipsoidal, 1.9–3.7 × 2.5–6.3 µm, hyaline becoming brown with days, thick walled.

Additional specimen examined—**ARGENTINA**, **Salta**, Parque Nacional El Rey, 25°13′59″S 64°42′58″W, 900 m asl, fungal garden of *Acromyrmex lundii*, Nov 2012, J.A. Marfetán E28 (UNQ E28; GenBank KU298288, KU298280).

Commentary—The subglobose vesicles of *E. atlas* easily separate it from *E. weberi, E. moelleri, E. microspora,* and *E. longivesica*. *Escovopsis atlas* is distinguished from *E. lentecrescens* by growth rate in PDA, from *E. aspergilloides* by smaller subglobose vesicles and conidia, and from *E. primorosea* by conidial coloration (pink) in mCYA.

Escovopsis catenulata Marfetán, A.I. Romero & Cafaro, **sp. nov.** Figs 7, 8e

MycoBank MB 816919

Differs from other *Escovopsis* species by its catenulate claviform vesicles.

Type—Argentina, Tucumán, Parque Nacional Campo de los Alisos, 27°13′48″S 65°54′51″W, 1500 m asl, fungal garden of *Acromyrmex aspersus*, Nov 2012, J.A. Marfetán E34 (**Holotype**, BAFC-H 52765; ex-type culture UNQ E34; GenBank KU298287).

Etymology—from the Latin *catenulatus* "forming short chains" referring to the production of vesicles in chains.

Colonies in PDA, colony diameter 6.2–9.2 cm after 7 days, initially white in color becoming light brown in the older part of the colony, abundant aerial mycelium. Hyphae hyaline, cylindrical, smooth, 3.7–13.7 µm diameter, with Woronin bodies highly visible. Conidiophores terminal, hyaline, growing from the aerial mycelium, cylindrical, orthogonal branched. Vesicles solitary or catenulate (2-4), cylindrical, occasionally claviform, 15–75 × 6.2–22.5 µm. Conidiogenous cells phialidic, with rounded base, elongated neck and

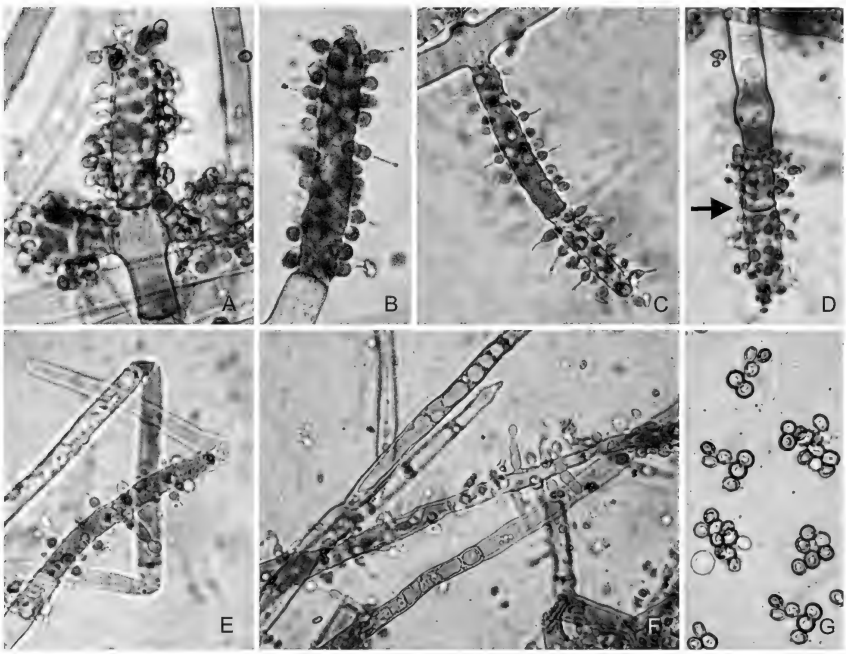

FIG. 7: *Escovopsis catenulata* (holotype, BAFC-H 52765). A. Conidiophore; B. Vesicles; C, D. Catenulate vesicles; E, F. Intercalary vesicle; G- Conidia.

inconspicuous collarette, 2.5–6.2 × 12.5–12.5 µm. CONIDIA catenulate, with basipetal development, subglobose to ovoid 1.22–6.25 × 1.6–6.25 µm, hyaline becoming brown later. Chlamydospores occasionally present in terminal chains, 7.95–17.4 × 12.3–17.3 µm.

ADDITIONAL SPECIMENS EXAMINED—**ARGENTINA**, **CORRIENTES**, Mercedes, 29°11′53″S 58°02′28″W, 98 m asl, fungal garden of *Acromyrmex lobicornis*, Mar 2008, J.A. Marfetán E17 (UNQ E17; GenBank KU298285, KU298284). **SANTA FE**, San Cristobal, 30°22′55″S 61°14′20″W, 67 m asl, fungal garden of *Atta vollenweideri* Forel, Aug 2008, J.A. Marfetán E18 (UNQ E18; GenBank KU298295); San Cristobal, 30°22′57″S 61°14′16″W, 67 m asl, fungal garden of *A. heyeri*, Aug 2008, J.A. Marfetán E19 (UNQ E19; GenBank KU298286). **BUENOS AIRES**, La Plata, 34°54′39″S 57°56′05″W, 10 m asl, fungal garden of *Acromyrmex lundii*, Apr 2009, J.A. Marfetán E7 (UNQ E7; GenBank KU298282).

COMMENTARY—*Escovopsis catenulata* is readily separated from *E. aspergilloides, E. lentecrescens,* and *E. atlas* by the absence of globose or subglobose vesicles. It is distinguished from *E. weberi, E. pseudoweberi, E. longivesica, E. microspora,* and *E. moelleri* by the presence of catenulate vesicles. Finally, *E. catenulata*

is separated from *E. primorosea,* which also presented catenulate cylindrical vesicles, by conidia coloration (pink in *E. primorosea*) in mCYA. Differentiation from *E. aspergilloides, E. kreiselii, E. lentecrescens, E. longivesica, E. microspora, E. moelleri,* and *E. primorosea* can be done using TEF1 and 28S rDNA sequences.

Escovopsis weberi J.J. Muchovej & Della Lucia, Mycotaxon 37: 192, 1990. FIG. 8B

Although our isolates identified as *E. weberi* agreed with the description by Muchovej & Della Lucia (1990), they showed some differences in size of particular structures. The vesicles in our isolates are wider (5–25 × (6.25–) 25–66.25 µm) than previously described (11–14 × 43–58 µm), and the conidia size range is also wider (2.5–5 × 2.5–6.25 µm) than the original description (2–3 × 2.2–3.3 µm). These data could represent normal morphological variation within the species; thus, we conservatively assigned our isolates to *E. weberi.*

SPECIMENS EXAMINED—**ARGENTINA**, **SANTA FE**, San Cristóbal, 30°22′54″S 61°14′15″W, 67 m asl, (UNQ E16; GenBank KU298308). **LA PAMPA**, Santa Rosa 36°36′58″S 64°18′51″W, 177 m asl (UNQ E22; GenBank KU298304); Toay, 36°32′42″S 64°03′01″W, 174 m asl (UNQ E26; GenBank KU298305). **SALTA**, El Rey, , 25°13′59″S 64°42′48″W, 900 m asl, (UNQ E31; GenBank KU298292); , 25°13′61″S 64°42′53″W, 900 m asl, (UNQ E41; GenBank KU298294).

Key to species of *Escovopsis*

1a. Conidiophores with vesicles *2*
1b. Conidiophores without vesicles 11

2a. Pink coloration in modified CYA media after 4 days ..... *E. primorosea*
2b. Brown coloration in modified CYA media after 4 days 3

3a. Subglobose or globose vesicles present 4
3b. Subglobose or globose vesicles absent 6

4a. Colony growth ≤0.7 cm diam after 14 days ..... *E. lentecrescens*
4b. Colony growth ≥0.7 5

5a. Subglobose vesicles (8.75–27.5 × 16.25–31.25 µm) ..... *E. atlas*
5b. Globose to subglobose vesicles (20 × 35 µm) ..... *E. aspergilloides*

6a. Catenulate vesicles present ..... *E. catenulata*
6b. Catenulate vesicles absent 7

7a. Conidia 3–3.5 × 7–10 µm ..... *E. moelleri*
7b. Conidia <7 µm long 8

8a. Vesicle >70 µm long ..... *E. longivesica*
8b. Vesicle <70 µm long 9

9a. Vesicle up to 6–7 µm diam, conidia 1.5–3 × 2–3 µm ..... *E. microspora*
9b. Vesicle generally bigger 10

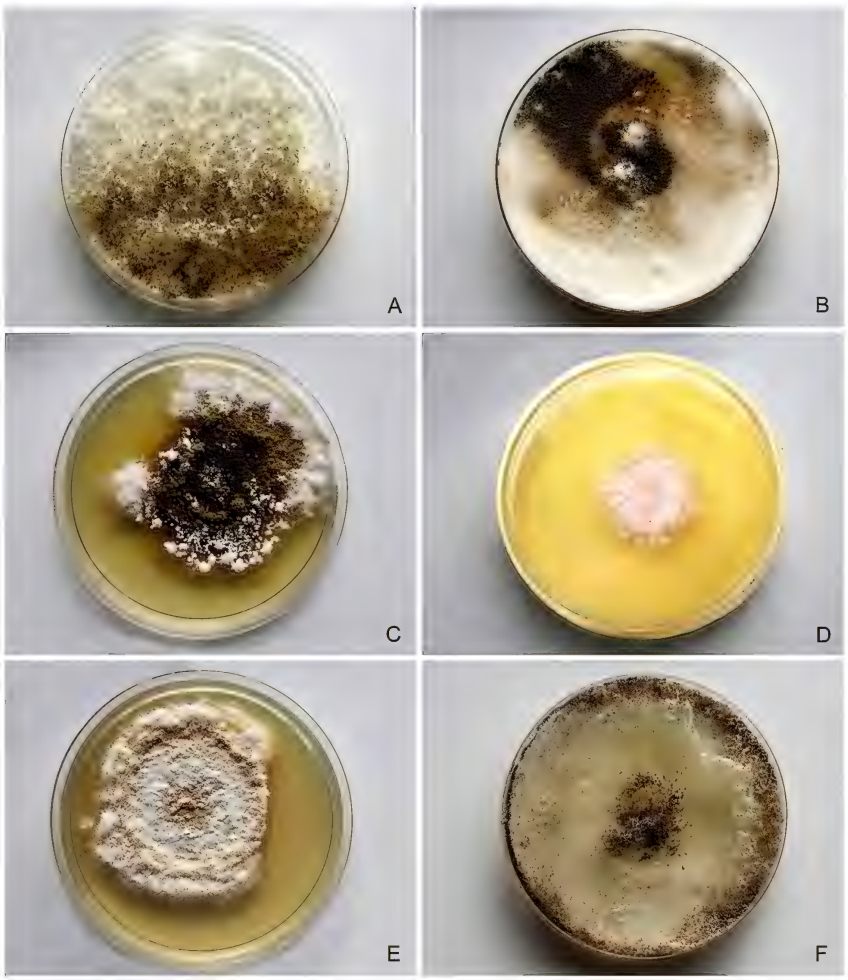

FIG. 8: Comparison of cultural characteristics of the six sequenced species in modified CYA media after 4 days: A. *Escovopsis longivesica*; B. *E. weberi;* C. *E. atlas*; D. *E. primorosea*; E. *E. catenulata*; F. *E. pseudoweberi.*

10a. Vesicles 5–20 µm diam, conidia 0.97–2.71 × 1.25–4.22 µm ..... *E. pseudoweberi*
10b. Vesicles 5–20 µm diam, conidia 2–5 × 2.2–6.25 µm ..... *E. weberi*
11a. Percurrent conidiogenous cells present ..... *E. kreiselii*
11b. Conidiophores *Trichoderma*-like; yellow soluble pigment present in PDA..... *E. trichodermoides*

Discussion

In Argentina, *Escovopsis* isolates have been reported from Santa Fé, Misiones, and Chaco Provinces (Folgarait & al. 2011; Taerum & al. 2007, 2010) and *E. weberi* isolates from Corrientes, Buenos Aires, and Santa Fé Provinces (Folgarait & al. 2011, Marfetán & al. 2015), but the scope of these studies focused on ecological and biological issues rather than taxonomy. Ours is the first study that describes *Escovopsis* isolates from Argentina at the species level using morphological and molecular data. Furthermore, it represents the most complete survey to date in Argentina (26 isolates) and one with the highest number of isolates spanning wide geographic (6 provinces) and host ranges (6 leaf-cutting ant species).

All the described species are well delimited by characters with clear diagnostic features that allow separation among species. Vesicle morphology and size and conidial shape and size are, as in other *Escovopsis* species, the most useful key characters. Additionally, we propose colony coloration in mCYA as a new diagnostic character within the genus.

Phylogenetic analyses using TEF1 and 28S rDNA presented robust results showing well supported clades for *Escovopsis longivesica* and *E. primorosea* isolates, supporting the hypothesis that these isolates belong to new species. The same analyses place *E. atlas, E. catenulata,* and *E. pseudoweberi* in the same clade with previously described species (Fig. 2) including *E. weberi* and *E. microspora* (Fig. 1). Although our results support the proposal of new *Escovopsis* species, it is clear that the molecular markers used are not enough to resolve species relationships. The lack of resolution using 28S rDNA and TEF1 genes is not new for *Escovopsis* species separation (Augustin & al. 2013). Previous work showed that these markers were unable to separate *E. weberi* and *E. microspora* (Augustin & al. 2013) even though in our 28S rDNA analysis we indeed were able to distinguish both species (Fig. 1). Limited taxon sampling may explain previous results for the non-Argentine species. Furthermore, this study confirmed that these markers are highly conserved and failed to resolve the phylogenetic relationships between some *Escovopsis* species, thus warranting evaluation of additional molecular markers.

Our data (Fig. 2) showed that the brown conidia clade was formed by *E. weberi* (ex-type), *E. aspergilloides, E. microspora, E. moelleri, E. lentecrescens*, and other unidentified associated isolates, while the white, pink, and yellow lineages were more closely associated with *Escovopsioides* than with *Escovopsis*. These results agreed with previous works (Masiulionis & al. 2015, Meirelles & al. 2015).

Surprisingly, our results showed that *E. trichodermoides* and *E. kreiselii* are more related to the white and pink conidiated clade than with the brown conidiated species (Fig. 2). In this sense, these two species have marked differences with the rest. Both species present holoblastic solitary conidia and conidiophores without vesicles (Masiulionis & al. 2015, Meirelles & al. 2015). These results could suggest that undescribed strains/isolates in these clades (pink, yellow, and white conidiated) could present additional differences to the known *Escovopsis* species. It is necessary to evaluate taxonomically the undescribed strains/isolates in these three clades to create a formal description of these interesting isolates and estimate their morphological variability in the genus. Moreover, in future studies scanning electron microscopy might be used to evaluate if there is any difference between the conidial ontogeny seen in *E. trichodermoides* and in the conidiogenous cells of the remaining species.

The present data showed no species-specific association between ant species and *Escovopsis* species, agreeing with previous works including bioassays showing that *Escovopsis* species can parasitize more than one leaf-cutting ant species (Folgarait & al. 2011, Taerum & al. 2007). In our study, *E. weberi* and *E. longivesica* were both isolated from *A. lundii* and *A. striatus* nests, indicating that these two species can infect different ant species. The same seems true for *E. atlas* isolated from *A. lundii* and *A. aspersus,* while *E. catenulata* and *E. pseudoweberi* were isolated from several different ant species. Furthermore, *E. catenulata* was isolated from ants in different genera, *Atta vollenweideri* and four *Acromyrmex* ant species contradicting any kind of specificity.

Our phylogenetic analyses (Fig. 2) did not show a particular phylogeography pattern among *Escovopsis* species and isolates. Although species from Argentina appear to be more closely related to species from Brazil than from other parts of South America, they are more closely associated with ant groups than with geographical site. However, our results suggest that temperate areas (southern Argentina) have a more restricted *Escovopsis* diversity (2 species) than in tropical zones (northern Argentina, Brazil, and Panama, with 11 species and several undescribed isolates). Species distribution studies, including an exhaustive identification of *Escovopsis* isolates, are needed to better understand which factors influence diversity and distribution and to build stronger hypotheses about its biogeography.

Evidence for additional species can be found in this work and others (Augustin & al. 2013) in comparing *E. weberi* isolates to the original description by Muchovej & Della Lucia (1990). There are still unidentified Argentine *Escovopsis* strains (data not shown) that are hardly distinguishable

from *E. weberi*. Finally, *Escovopsis* species are well delimited by morphological character correlation. However, for some taxa, the molecular markers used in this study have proved inadequate in satisfactorily solving the phylogenetic relationships at the species level. Further research on this matter is needed to develop a robust phylogenetic framework for *Escovopsis*.

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Rosellinia angusta and *R. menglana* spp. nov. and two new *Rosellinia* records from China

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Abstract—Two new species, *Rosellinia angusta* and *R. menglana*, are described, and *R. bonaerensis* and *R. longispora* are recorded as new to China.

Key words—*Ascomycota*, pyrenomycetous fungi, taxonomy, *Xylariaceae*

Introduction

Here we describe two new species of *Rosellinia*, and two new *Rosellinia* records from China. These specimens are conserved in the Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS).

Materials & methods

The specimens were examined using a Motic SMZ-168 stereomicroscope. Microscopic features of asci and ascospores were described from slide preparations mounted in water and Melzer's reagent, and measurements were made from 20 ascospores, using a Zeiss Axioskop 2 plus compound microscope. Stromata were photographed using a Sony NEX-7 digital camera.

Taxonomy

Rosellinia angusta Wei Li bis & L. Guo, **sp. nov.** Figs 1–3

Fungal Names FN 570568

Differs from *Rosellinia floridana* by its larger ascospores and usually with narrowly rounded or tapering ends.

Type: China, Yunnan, Puer, Simao, alt. 1260 m, on corticated wood, 18.III.2017, L. Guo 12234 (**Holotype**, HMAS 247749).

Etymology: The epithet refers to the narrowly rounded or tapering ends of the ascospores.

Subiculum felted, brown, evanescent at maturity. Stromata subglobose, solitary to gregarious, dark brown, 700–1000 × 800–1000 µm. Ostioles papillate, black. Ectostroma 60–80 µm thick, black. Entostroma disappearing in old stage. Perithecia subglobose, 600–700 × 700–800 µm. Ascus apical ring bluing in Melzer's iodine reagent, 9–13 µm tall, upper diameter 4–5 µm, lower 4–6 µm. Ascospores asymmetrically ellipsoidal, usually with narrowly rounded or tapering ends, sometimes papillate or broadly rounded, 22–30 × 7–11 µm, dark brown, with straight germ slit as long as spore.

Additional specimens examined: **China**, **Zhejiang**, **Wencheng**, Shimen, alt. 520 m, 22.XI.2016, L. Guo 12027 (HMAS 247757); L. Guo 12037 (HMAS 255301); **Fujian**, **Qinning**, Qingliu, alt. 600 m, 27.VII.1960, Q.Z. Wang & al. 528 (HMAS 29554); **Guizhou**, **Libo**, Maolan, alt. 550 m, 12.IX.2013, W. Li 2417 (HMAS 253043) ; W. Li 2426 (HMAS 253041).

Comments: *Rosellinia angusta* is similar to *R. floridana* L.E. Petrini, which differs in its smaller ascospores (19–26 × 8–10 µm) with broadly rounded ends (Petrini 2013a,b).

Rosellinia menglana Wei Li bis & L. Guo, **sp. nov.** Figs 4–6

Fungal Names FN 570569

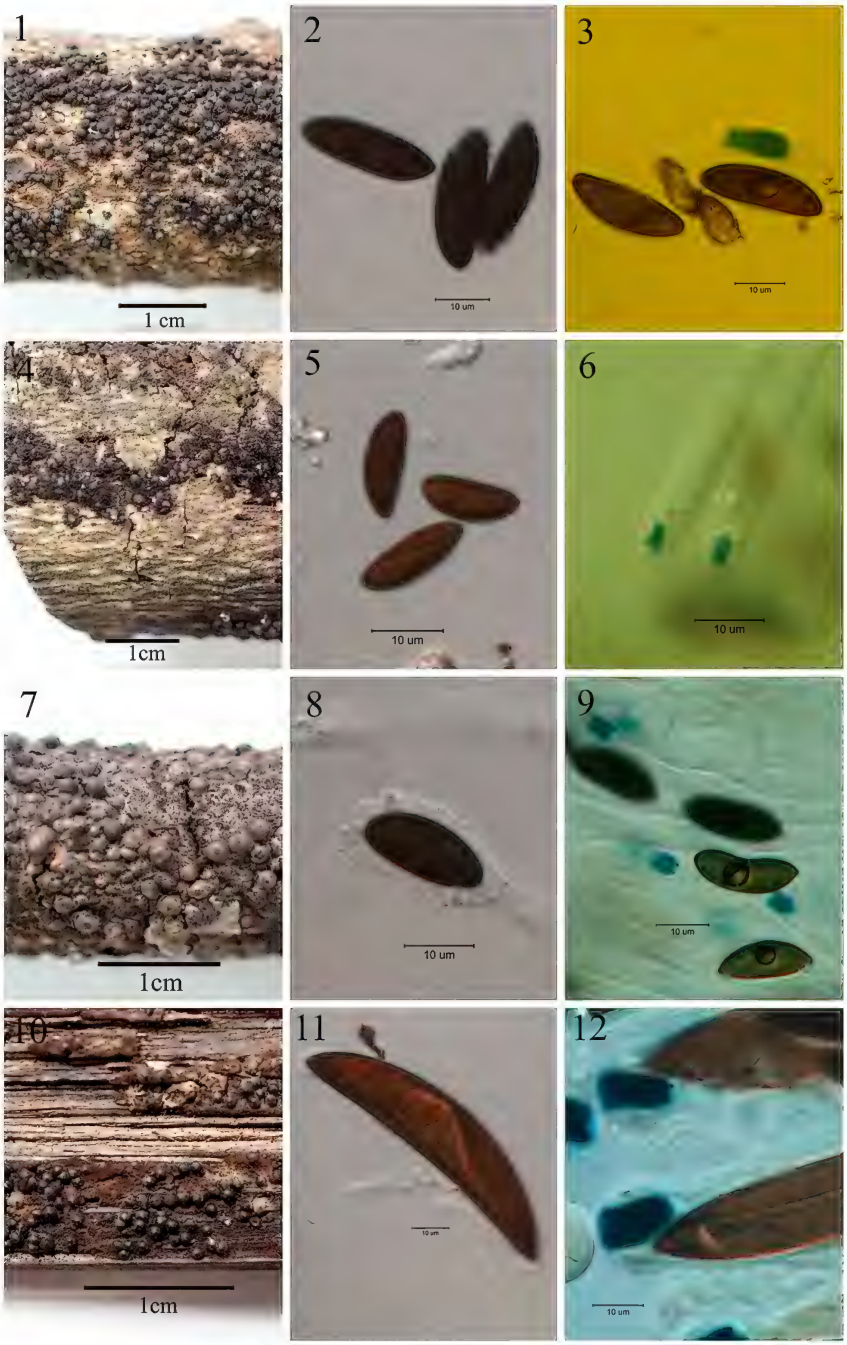
Differs from *Rosellinia sigmoidea* by asymmetrically ellipsoidal ascospores with sigmoid germ slit extending over the whole spore length, and its well developed subiculum.

Type: China, Yunnan, Mengla, alt. 750 m, on wood, 17.X.2013, W. Li 2989 (**Holotype**, HMAS 245216).

Etymology: The epithet refers to the type locality, Mengla.

Subiculum well developed, roughly silky, dark brown, retained at maturity. Stromata solitary to densely gregarious, subglobose, dark brown, 700–900 × 800–1000 µm, almost completely embedded in the subiculum. Ostioles papillate, dark brown. Ectostroma 80–140 µm thick, black. Entostroma white, disappearing in old stage. Perithecia 500–700 × 660–800 µm. Ascus apical ring bluing in Melzer's iodine reagent, 3.5–4 µm high, upper diameter 2.5–3 µm,

Figs 1–12. ***Rosellinia angusta*** (HMAS 247749, holotype). 1. Stromata on wood; 2. Ascospores; 3. Ascospores and ascus apical ring. ***Rosellinia menglana*** (HMAS 245216, holotype). 4. Stromata on wood; 5. Ascospores; 6. Ascus apical rings. ***Rosellinia bonaerensis*** (HMAS 255304). 7. Stromata on wood; 8. Ascospore; 9. Ascospores and ascus apical rings. ***Rosellinia longispora*** (HMAS 26813). 10. Stromata on wood; 11. Ascospore; 12. Ascospores and ascus apical rings.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lower 1.5–3 μm. Ascospores asymmetrically ellipsoidal, with narrowly rounded ends, 13–20 × 5–8 μm, brown, with sigmoid germ slit as long as spore.

Comments: *Rosellinia menglana* is similar to *R. sigmoidea* Q.R. Li & al., which differs in its ellipsoidal ascospores with the germ slit extending the half length of the spore and its sparse subiculum (Li & al. 2015).

Rosellinia bonaerensis Speg.,
Anales Mus. Nac. Hist. Nat. Buenos Aires 6: 258, 1898. Figs 7–9

Subiculum woolly, brown to red-brown, retained at maturity. Stromata solitary to gregarious, subglobose to semiglobose, with flat top and short stipe on base, 900–1300 × 1100–1500 μm, with lower part embedded in the subiculum, dark brown to black. Ostioles papillate, black. Ectostroma 100–120 μm thick, black. Entostroma dark brown, retained at the base at maturity. Perithecia 800–1100 × 900–1200 μm. Ascus apical ring bluing in Melzer's iodine reagent, 5–8 μm tall, diameter 3.5–5 μm above, 3–5 μm below. Ascospores asymmetrically ellipsoidal, 17–23 × 7–9 μm, entirely surrounded by a slimy sheath, with a cellular appendage at one end, 1–2 × 1.5–2 μm, dark brown, with straight germ slit almost as long as spore.

Specimen examined: **CHINA**, **Shandong**, **Zaozhuang**, Bodugu, alt. 250 m, on wood, 22.X.2017, L. Guo 12256 (HMAS 255304).

Comments: Our specimen is morphologically similar to the holotype of *R. bonaerensis,* but their ascus apical rings were slightly taller than those of the holotype (4.5–5.5 μm; Petrini 2013).

Rosellinia longispora Rick, Brotéria Ci. Nat. 1: 189, 1932. Figs 10–12

Subiculum felted, brown to dark brown, retained at maturity. Stromata gregarious or solitary, conical, 500–800 × 600–900 μm, surface smooth, black. Ostioles papillate, black. Ectostroma 80–100 μm thick, black. Entostroma disappearing at maturity. Perithecia subglobose, 700–800 μm high, 800–900 μm diam. Ascus apical ring dark bluing in Melzer's iodine reagent, 12–20 μm tall, diameter 6–10 μm above, 5–10 μm below. Ascospores asymmetrically ellipsoidal, with rounded or pinched ends, 56–97 × 12–20 μm, light brown to dark brown, with slimy sheath at one side and both ends, 1 μm thick, with straight germ slit as long as spore.

Specimen examined: **CHINA**, **Guangxi**, **Wude**, on decayed wood, 21.IX.1956, Z.C. Liang 747 (HMAS 26813).

Comments: Our specimen is morphologically similar to the description of holotype of *R. longispora*, but its subiculum retained at maturity, not

evanescent; and ectostromata were slightly thicker than those of the holotype (25–75 µm; Petrini 2013b).

Discussion

Including the four species reported in this paper, we now recognize 45 *Rosellinia* species in China (Teng 1963; Tai 1979; Ju & Rogers 1990, 1999; Yuan & Zhao 1993; Lu & al. 2000; Liu & al. 2010; Petrini 2013a,b; Li & Guo 2015, 2016, 2018; Li & al. 2015; Su & al. 2016).

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Phaeonectriella alba sp. nov. from the River Nile, Egypt

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Abstract— A new species, *Phaeonectriella alba,* collected from the River Nile, Aswan Governorate, Egypt, is described and illustrated. Phylogenetic analyses of LSU rDNA sequences placed the new species with *P. lignicola* (the type species) in a highly supported clade. *Phaeonectriella alba* is characterized by conical, superficial, hyaline, membranous ascomata; 4- or 8-spored asci that are persistent, clavate, with a truncate apical thickening and a simple pore and cytoplasmic retraction below apex; early deliquescing catenophyses; and ascospores that are ellipsoid to broad ellipsoid, thin-walled, 1–3 septate, hyaline when young, becoming brown at maturity, with an apical germ pore in each cell and without appendages.

Key words—freshwater fungi, *Halosphaeriaceae*, *Microascales*, Middle East, subtropical fungi

Introduction

During an ongoing study of freshwater fungi colonizing herbaceous and woody material submerged in water around four islands in the southern part of the River Nile, Egypt, an undescribed species of *Phaeonectriella* R.A. Eaton & E.B.G. Jones was discovered. The genus was established to accommodate *P. lignicola* R.A. Eaton & E.B.G. Jones that was described from wooden blocks placed for 54 weeks in a cooling tower in Wales (Eaton & Jones 1970). *Phaeonectriella lignicola* was later recorded from test blocks exposed in water

[†] We regret to announce the sudden death of Prof. Ahmed M. Abdel-Raheem during the course of this work.

cooling towers in India (Natarajan & Udaiyan 1978) and from freshwater habitats in Taiwan (Jones 1995). Ascospore appendages were not mentioned in the original description of *P. lignicola* or the collection from Taiwan (Eaton & Jones 1970, Jones 1995), but Hyde & al. (1999) later identified *P. lignicola* from Mauritius that differed by having polar appendages and lacking polar germ pores. Hyde & al. (1999) also described a second species, *P. appendiculata* K.D. Hyde & al. from submerged wood in freshwater habitats in Australia and Philippines, with polar appendages and larger-sized ascospores. Jones (1995) assigned *P. lignicola* to *Halosphaeriaceae* after observing typical halosphaeriaceous pseudoparenchyma in the centrum that form catenophyses, and asci with an apical pore in the isolate from Taiwan. No molecular data are available for *P. appendiculata* or for the Mauritius specimen of *P. lignicola*.

Phaeonectriella has common morphological characters with the genus *Aniptodera* Shearer & M.A. Mill., including: hyaline ascomata; persistent asci with a truncate apical thickening and a simple pore and cytoplasmic retraction below apex; and ascospores with or without polar appendages. *Phaeonectriella* has thin-walled ascospores that become brown at maturity, with an apical germ pore and with or without polar appendages. In contrast, *Aniptodera* sensu stricto species have thick-walled ascospores that are hyaline and with or without polar appendages (Eaton & Jones 1970, Shearer & Miller 1977, Shearer & Crane 1980, Koch 1982, Shearer 1989). Another morphologically related genus is *Halosarpheia* Kohlm. & E. Kohlm. which has dark-colored ascomata; thin-walled asci that deliquesce early; and bi-celled, ellipsoidal, hyaline, thin-walled ascospores with polar unfurling appendages (Kohlmeyer & Kohlmeyer 1977). Molecular studies of the ribosomal genes suggested that *Aniptodera* and *Halosarpheia* are polyphyletic, and several new genera were named to accommodate phylogenetically distant taxa from the type species (Campbell & al. 2003; Pang & al. 2003; Jones & al. 2015, 2017). *Phaeonectriella lignicola* was placed in a distant clade from the type species of the two genera: *Aniptodera chesapeakensis* Shearer & M.A. Mill. and *Halosarpheia fibrosa* Kohlm. & E. Kohlm. (Pang & al. 2003, this study).

Materials & methods

Sample collection

Submerged decayed culms of *Phragmites australis* (Cav.) Trin. ex Steud. (*Poaceae*) were collected from the River Nile in Aswan Governorate. Collected samples were placed in clean plastic bags and returned to laboratory. Samples were examined under

a stereomicroscope upon return to the laboratory, placed in sterile humid plastic boxes for incubation, and examined periodically over three months. Ascomatal squash of the new species was mounted in freshwater for all measurements and photography. Photographs were taken using an Olympus BX51 differential interference contrast light microscope and Optika view version 7.3.1.7 digital imaging system. Vertical sections of ascomata were prepared using Leica CM1100 cryostat. Herbarium materials of the new species were prepared by drying natural wood bearing the fungus at 60°C for 24 h. The herbarium material of the new species was deposited at Assiut University Mycological Centre, Assiut, Egypt (AUMC). An identifier number was registered (MycoBank 2017). Ascospores of the new species did not grow in culture.

DNA extraction, sequencing, and phylogenetic analysis

DNA was extracted directly from fruiting structures by collecting the ascomata from the surface of the natural wood carefully using clean, sterilized fine forceps. Ascomata were washed twice in sterile distilled water by centrifugation at 10000 rpm for 1 minute. Microbial DNA Extraction Kit was used for DNA extraction according to the manufacturer's instructions. Partial nuclear LSU rDNA sequence was amplified with the primers LROR and LR7 (Vilgalys & Hester 1990, Bunyard & al. 1994). Our attempts to obtain ITS and SSU rDNA sequences of the new species were unsuccessful. PCR reactions were carried out by Macrogen Inc., Korea using MGTM Taq-HF DNA polymerase. The cycling parameters were: initial denaturation at 96°C for 3 min; 35 cycles of 96°C for 15 s, 52°C for 45 s, 72°C for 1 min 30 s; and final elongation at 72°C for 7 min. The same PCR primers were used for sequencing. Sequencing procedures used were as previously described (Abdel-Wahab & al. 2009, 2016). The nuclear LSU rDNA sequence of the new taxon was deposited at GenBank (Fig. 1). The sequence was aligned with *P. lignicola,* closely related genera in the *Halosphaeriaceae*, and representatives of *Microascaceae* and *Xylariales* using ClustalX (Thompson & al. 1997) and optimized manually. Maximum-parsimony (MP) and Maximum-likelihood (ML) phylogenetic analyses were carried out using PAUP* 4.0 (Swofford 2002). Maximum-likelihood (ML) analysis (Felsenstein 1981) was performed using heuristic searches with random stepwise addition of 100 replicates and TBR rearrangements. The best optimal model of nucleotide substitution for the ML analyses was determined using hierarchical likelihood ratio tests as implemented in Modeltest 3.7 (Posada & Crandall 1998). TrN was the best fit for LSU rDNA dataset. Bayesian phylogenetic analysis was performed in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) with the GTR model determined using MrModeltest 2.3 (Nylander 2004). Phylogenetic trees were visualized using NJplot (Perrière & Gouy 1996) and edited using Adobe Illustrator CS6.

Phylogenetic results

The LSU rDNA dataset comprised 47 sequences: 41 from *Halosphaeriaceae* and three from *Microascaceae,* with an outgroup of three from *Xylariales* (Fig. 1). The maximum parsimony dataset consisted of a total of 862

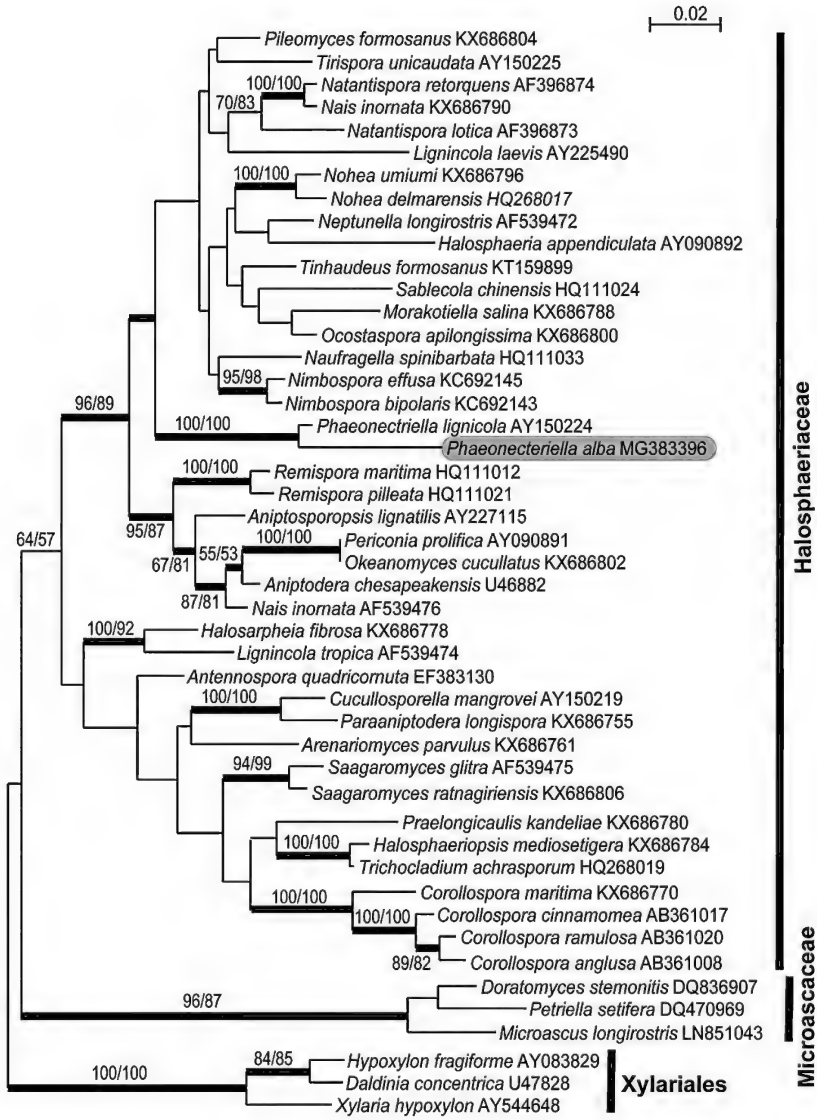


FIG. 1. Phylogenetic relationships of *Phaeonectriella alba* based on nucleotide sequences of the LSU rDNA along with the type species *P. lignicola* and other related genera in the *Halosphaeriaceae* and the closely related family *Microascaceae*. Representatives of the order *Xylariales* were used as outgroup. The maximum likelihood tree (ML) (–ln likelihood = **14607.49**) was constructed as described in the text. Bootstrap support on the nodes represents ML and MP ≥50%. Branches receiving Bayesian PP ≥95% are in bold. The sequence of the new species is in the grey box.

characters, of which 537 were constant, 83 variable and parsimony-uninformative, and 242 were counted as parsimony-informative. Maximum Parsimony analyses resulted in 18 most parsimonious trees, all of which had an equal tree length of 1105 steps, a consistency index of 0.4416, a retention index of 0.6672, and a rescaled consistency index of 0.2947. Maximum likelihood analysis yielded one tree (–ln likelihood = 14607.49; Fig. 1), and Bayesian analysis yielded trees with similar overall topology. The new species *P. alba* grouped consistently with the type species, *P. lignicola,* in all analyses with 100% statistical support in ML/MP and BYPP.

Taxonomy

Phaeonectriella alba Abdel-Wahab & Abdel-Aziz, **sp. nov.** Figs 2–13

MycoBank MB 823352

Differs from *Phaeonectriella lignicola* by its smaller, hyaline, conical, superficial ascomata, its shorter, wider asci, and its larger, symmetrical ascospores.

Type: Egypt, Aswan (24°05′42″N 32°53′06″E), in the River Nile, on decayed submerged culms of *Phragmites australis*, 3 January 2014, coll. Ahmed E. Abdel-Aziz (**Holotype**, AUMC-12004-H; GenBank MG383396).

Etymology: Latin *alba* = white, the color of the ascomata.

Saprobic on decayed submerged stem of *Phragmites australis*.

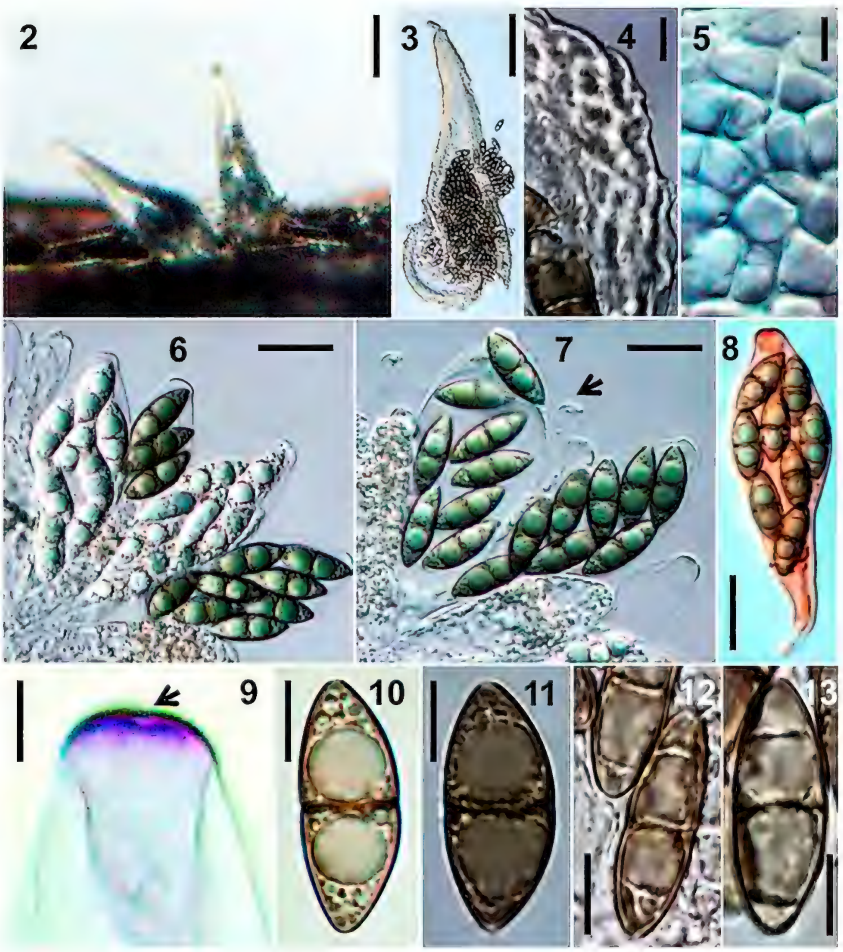
Sexual morph: Ascomata 200–325 µm long × 160–205 µm wide (mean = 251 × 176 µm, n = 5), conical, superficial, hyaline, membranous, solitary, ostiolate. Neck 175–225 µm long × 50–75 µm wide, periphysate, ostiolar canal 82.5 × 12.5 µm. Peridium 12–27 µm thick, one-layered, hyaline, comprising 6–9 cell layers, thick-walled, forming a textura angularis. Catenophyses early deliquescing. Asci 77–138 × 28–41 µm (mean = 99 × 34 µm, n = 30), clavate, unitunicate, persistent, 4 or 8-spored, apically truncated, thickened, with apical simple pore and cytoplasm retraction below the apex. Ascospores 25–33 × 8–13 µm (mean = 29 × 11 µm, n = 50), ellipsoid to broad ellipsoid, overlapping biseriate to multiseriate, hyaline when young, pale brown to brown at maturity, thin-walled, bi-celled, symmetrical, not constricted at the septum, with polar germ pores and without appendages.

Asexual morph: Undetermined.

Comments—Table 1 compares the morphology of *Phaeonectriella alba* and the other *Phaeonectriella* species. The type species *Phaeonectriella lignicola* is the most similar to *P. alba* but differs by its larger, hyaline to light-brown, globose to subglobose, immersed ascomata; its longer, narrower asci; and its smaller, longitudinally asymmetrical ascospores (Eaton & Jones 1970).

Table 1. Comparison of the morphology of *Phaeonectriella* species.

Fungus	Ascomata	Neck	Cateno-physes	Peridium	Asci	Ascospores
P. alba (this study)	200–325 × 160–205 µm, conical, superficial, hyaline	175–225 × 50–75 µm, periphysate	Early deliquescing	Forming a textura angularis	77–138 × 28–41 µm, 4- or 8-spored, apically truncate & thickened, with apical pore & cytoplasm retraction below the apex	25–33 × 8–13 µm, ellipsoid to broad ellipsoid, hyaline when young, (pale) brown when mature, symmetrical, not constricted at the septum, with polar germ pores, without appendages
P. appendiculata (Hyde & al. 1999)	150–300 µm diam, sub-globose, all to partly immersed, hyaline	No data	Not seen	Forming a textura angularis	100–140 × 24–30 µm, 8-spored, thin-walled, apically truncate & thickened, with apical pore & cytoplasm retraction below the apex	32–42 × 10–12 µm, fusiform, hyaline or pale brown, not constricted at the septum, with polar appendages
P. lignicola (Eaton & Jones 1970)	100–575 × 100–350 µm, globose to sub-globose, immersed, hyaline to pale brown	100–317 × 46–93 µm, periphysate	Not seen	Cells homogenous	100–417 × 22–35 µm, 8-spored, with apical pore	26–30 × 9.5–11 µm, ellipsoidal, hyaline when young, grey to brown when mature, asymmetrical, with polar germ pore in each cell
P. lignicola (Sensu Hyde & al. 1999)	100–575 µm diam, sub-globose, immersed, hyaline to pale brown,	No data	Not seen	Cells compressed	100–120 × 22–35 µm, 8-spored, apically truncate & thickened, with apical pore & cytoplasm retraction below the apex	26–30 × 9.5–11 µm, ellipsoidal-fusiform, hyaline or pale brown, grey-brown when mature, longitudinally asymmetrical, septum slightly constricted, with polar appendages



Figs 2–13. *Phaeonectriella alba* (AUMC-12004-H, holotype): 2. Superficial ascomata on wood. 3. Vertical section through ascoma. 4. Vertical section through peridium showing thick-walled hyaline cells forming a textura angularis. 5. Surface view of peridial cells. 6–8. Asci at different stages of maturity (7. Apical simple pore (arrowed). 8. Ascus stained in Congo Red). 9. Apical part of ascus stained in Toluidine blue showing the simple pore (arrowed), truncate apical thickening and cytoplasmic retraction below apex. 10–13. Variously shaped ascospores. Scale bars: 2 = 140 μm; 3 = 100 μm; 4, 5, 9–13 = 10 μm, 6–8 = 20 μm.

Phaeonectriella appendiculata differs from *P. alba* by its globose to subglobose, immersed to erumpent ascomata; its smaller asci; and its larger ascospores with polar appendages (Hyde & al. 1999).

Ascospore appendages were not mentioned in the holotype of *P. lignicola* nor in the Taiwanese specimen (Eaton & Jones 1970, Jones 1995). However, the specimen from Mauritius identified as *P. lignicola* by Hyde & al. (1999) did produce polar appendages. This specimen should perhaps be designated as "*Phaeonectriella lignicola*" sensu Hyde & al. (1990), pending a molecular study to resolve whether or not polar appendages are typical of *P. lignicola*.

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New records of myxomycetes from the Comoros Islands

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Abstract—In a 2016 study encompassing four different regions and nine different localities on Ngazidja Island, ten myxomycete species were identified representing five genera: *Arcyria* (1 sp.), *Badhamia* (1 sp.), *Diderma* (1 sp.), *Didymium* (4 spp.), and *Physarum* (3 spp.). *Artocarpus heterophyllus*, the most productive substrate plant, yielded four myxomycete species, followed by *Ricinus communis* with two. The study methods and results of this research are outlined and accompanied by notes, comments, and microscopic images for selected species.

Keywords—Afrotropics, Indian Ocean, Mozambique Channel, volcanic

Introduction

The Comoros Islands lie in the Indian Ocean at 10–20°S 40–50°E at the northern entrance of the Mozambique Channel between Mozambique and the northern tip of Madagascar (Central Intelligence Agency 2017). This independent state consists of four volcanic islands—Ngazidja (Grande Comore), Anjouan (Ndzouani), Mayotte (Maoré), and Moheli (Mwali). Geochemical estimates place island formation at 1.4–15 million years ago (mya) for Mayotte, 0.62–2.8 mya for Moheli, 0.4–1.5 mya for Anjouan, and 0.13 mya to the present time for Ngazidja (Bachèlery & al. 2016; Emerick & Duncan 1982, 1983; Esson & al. 1970; Flower 1973; Nougier & al. 1986; Späth & al. 1996).

Ecologically, the Comoros Islands belong in the Afrotropical biogeographic realm and are characterized by very rich biotopes and high biodiversity

(Olson & Dinerstein 2002). Among the few studies of the Comoros flora, the most complete is that of Voeltzkow (1917), who cited 935 vascular plants. Most (84.4%) of the land surface is agricultural and 1.4% is forested (Central Intelligence Agency 2017). Endemic coastal and low elevation vegetation has been almost completely destroyed by anthropogenic effects (e.g., deforestation, agriculture, population density, and exotic species), but the forests seem to be better protected at high elevations (Wikipedia 2017a).

Comoros has a humid tropical climate, with the November–April rainy season followed by dry weather conditions during December–March. Temperatures range between 24 and 28°C, and the annual average precipitation is high (200 cm); due to the monsoon climate effects, there is abundant rain even during the dry season (Capaustral 2017).

Until recently, myxomycetes (traditionally treated as fungi) have been placed in *Eumycetozoa* (the true slime molds); recent molecular data, however, supports them in the *Amoebozoa* (Stephenson 2011). Myxomycetes have not previously been reported from this region (Ndiritu & al. 2009, Ndiritu & de Haan 2014); here we report the first records from Comoros.

Materials & methods

Collection sites—The study area was mainly Ngazidja (11°40′S 43°20′E), the largest and most densely populated (c. 400,000; Wikipedia 2017b) of the Comoros islands (Plate 1). The island covers 1862 km^2, and its elevations range from sea level to 2361 m at the summit of the volcano Karthala (Singaomara 2017). Our study was carried out mainly in five (of twelve) regions—Bambao, Dimani, Hamanvou, Oichili, and Mboudé. The climate of these regions is summarized in Plate 2 and Table 1.

Specimens—During the dry season (September) of 2016, we collected material randomly from five different regions on Ngazidja Island. In each locality, various substrates (aerial litter, ground litter, bark, coarse woody debris, rabbit dung) were placed in paper bags and documented (e.g., material type, dates of collection, locality, geographical coordinates, and elevation). Location coordinates were determined using a Garmino® eTrex Legend® HCx GPS. After placing sterile filter paper in sterile glass Petri dishes, sample material was placed on the bottom and were wetted with distilled water for 24–48 hours for the application of the moist chamber technique as described in Stephenson & Stempen (1994). Moist chamber cultures were prepared with 10 samples of the various substrates. Water was added every few days as required, to maintain the humidity for the entire observation period of up to four weeks. Each day stereomicroscopic (Nikon SM 800) observations and notes were taken; appearance and mature fructifications of myxomycetes were photographed. Then fructifications were pre-treated for slide-coverslip permanent preparations using Hoyer's medium. Light photomicrographs were obtained using

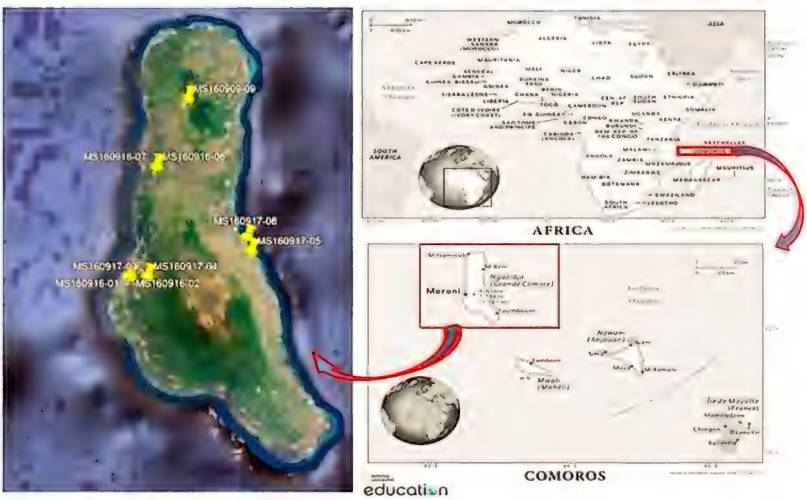

Plate 1. Localities sampled for myxomycetes on Ngazidja (Grand Comore)

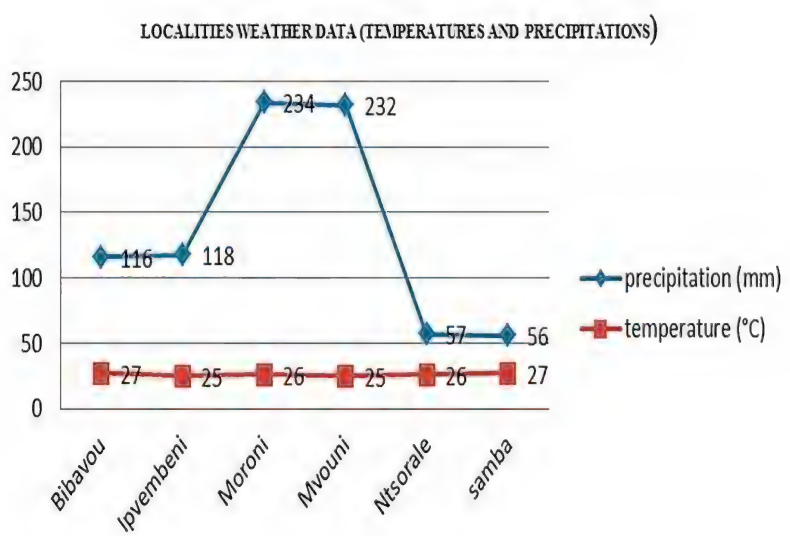

Plate 2. Average annual temperatures and precipitation for Bibavou, Ipvembeni, Moroni, Mvouni, Ntsorale, and Samba

a Nikon DS-Fi 1 microscope equipped with a Nikon Eclipse 50i camera system. Plasmodium, sporangium type, capillitium, peridium, spores, and other descriptive characters were recorded (Plates 3–12). Taxonomic position, nomenclature, and identification of species were determined by utilizing data obtained from stereo- and light-microscopical observations, published references, and internet sources (Ing 1999; Lado & Pando 1997; Lado 2001; Lakhanpal & Mukerji 1981; Farr 1976, 1981; Martin & Alexopoulos 1969; Martin & al. 1983; Nannenga-Bremekamp 1991; Stephenson & Stempen 1994; Thind 1977; Discover Life 2017; Global Biodiversity Information Facility 2017; Index Fungorum 2017). The specimens are deposited in the Uludag University Mycology Laboratory, Bursa, Turkey (UUML), and in Mohamed Seifou Youssouf's personal collection (MSY). Collections are cited using the collector initials (Mohamed Seifou [Youssouf] = MS), date (month, day, year), and number of collections. A map of the area, a climate diagram, and climatic data covering average temperature and precipitation in the study area from 2010 to 2016 are presented in Plates 1 and 2 and Table 1.

Table 1. Climate data* for Comoros Island localities

Localities	Precipitation (mm)	Temperature (°c)
Bibavou	116	27
Ipvembeni	118	25
Moroni	234	26
M'vouni	232	25
Ntsorale	57	26
Sambamadi	56	27

*Source: National Civil Aviation and Meteorological Agency of Comoros

Results & discussion

This first study of myxomycetes from Comoros revealed three families (*Arcyriaceae, Didymiaceae, Physaraceae*), five genera (*Arcyria, Badhamia, Diderma, Didymium, Physarum*), and ten species, all new records for the country. Nine species are also known from elsewhere in Africa.

Moist chamber cultures prepared with samples from *Artocarpus altilis, Artocarpus heterophyllus, Carica papaya, Litchi sinensis, Pterocarpus indicus,* and *Ricinus communis* were positive, but some materials from rotting wood of *Cocos nucifera,* from litter and twigs of *Terminalia catappa,* from rabbit dung were invaded by filamentous molds during incubation, and no species of myxomycetes species were obtained.

All myxomycete collections recorded in our study are listed below alphabetically according to genus and species. The abbreviation "cf." after *Badhamia affinis* indicates that the specimen could not be identified with certainty.

Species

Arcyria insignis Kalchbr. & Cook, Grevillea 10: 143 (1882) Pl. 3

Sporocarps gregarious or bundle or sometimes scattered, bright rose or pale rose usually with an orange color 0.5–1.5(–3) mm tall, short-stalked sporangia. Hypothallus inconspicuous. Stalk <0.2-0.7 mm high, reddish-orange, filled with subglobose or spore-like cells. Peridium remaining as shallow, saucer-shaped cup, radially wrinkled, the inner surface warted, often in a network. Capillitium of pale yellow to colourless close network of soft threads, meticulously attached to the calyculus, marked with rings and half rings, in part meticulously spinulose to nearly smooth and occasionally with a few bulbous free ends, 2–3µm diameter. Spores pinkish in mass, colorless in transmitted light, with scattered inconspicuous warts, 6–8µm diam. Plasmodium whitish.

Specimen examined—**COMOROS ISLANDS, Ngazidja Island, Moroni city,** vegetable garden: 11°43.26′S 43°15.12′E, 34 m elev., on *Artocarpus heterophyllus* debris, 16.09.2016, Mohamed Seifou (MSY 160916-01a).

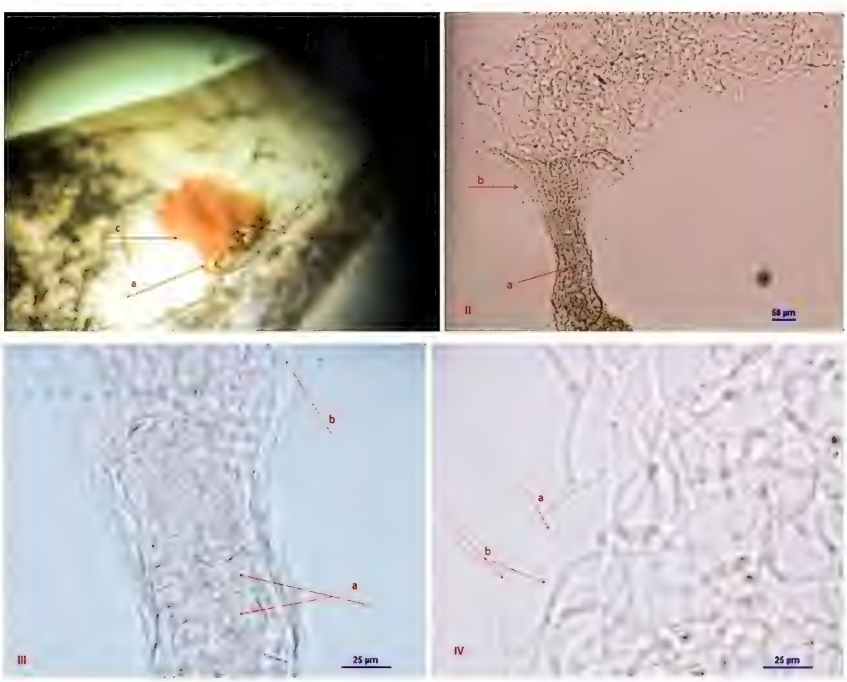

Plate 3. *Arcyria insignis* (MSY 160916-01a).
I- sporangium; a. stalk, b. calyculus, c. peridium; II- a. spore like-cells, b. calyculus;
III- a. spore like-cells, b. spores; IV- a. capillitium, b. spores.

Comments—*Arcyria insignis* is considered cosmopolitan (Martin & Alexopoulos 1969, Lakhanpal & Mukerji 1981) and has been reported previously from Africa (Ndiritu & al. 2009, Wrigley de Basanta & al. 2013, Discover Life 2017).

Badhamia cf. ***affinis*** Rostaf., Śluzowce Monogr.: 143 (1874) Pl. 4

Fructification gregarious, imbricate, depressed in the center and doughnut-shaped, blackish, mucilaginous, devoid of lime deposit. Hypothallus and stalk not observed. Peridium single and membranous, rugulose, translucent, minutely covered with aggregate material. Capillitial tubules conical, spike-like and smooth, not interconnected, sometimes bifurcate on the base and pointed end, radiating from a sponge-like plate and not reach for connecting to peridium, brownish, lime granules were not observed. Spores globose-subglobose, verruculose with wart cluster, free, brown in transmitted light, 12–14(–15) µm diameter.

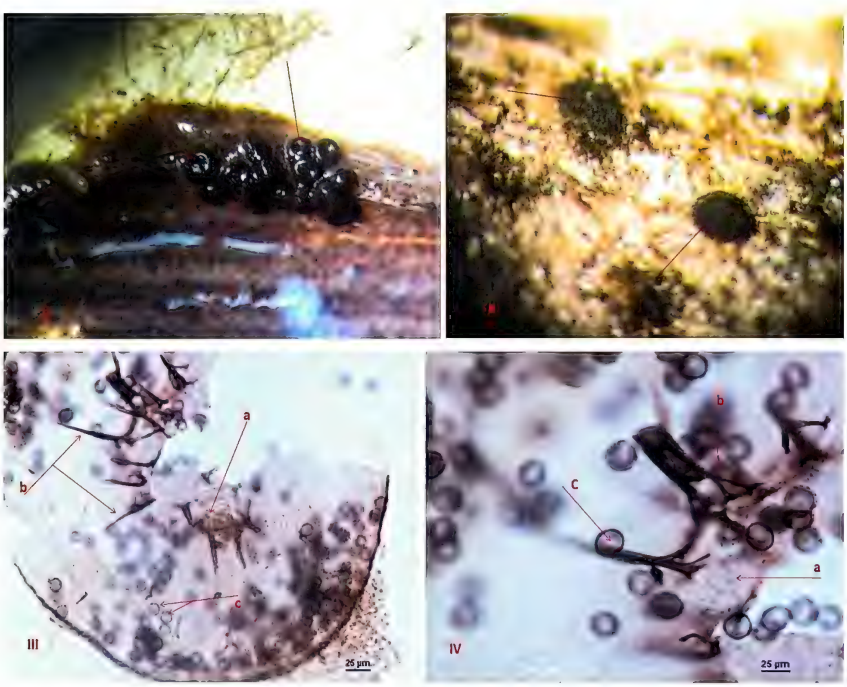

Plate 4. *Badhamia* cf. *affinis* (MSY 160916-01b).
I- sporocarps; II- dry sporocarps; III- a. sponge-like plate, b. capillitial tubules, c. spores; IV- a. sponge-like plate, b. capillitial tubule with a forked base.

Specimen examined—**COMOROS ISLANDS, Ngazidja Island, Moroni city,** vegetable garden: 11°43.26′S 43°15.12′E, 34 m elev., on *Artocarpus heterophyllus* debris, 16.09.2016, Mohamed Seifou (MSY 160916-01b).

Comments—This species shares some similarities with other species such as *Badhamia affinis, Badhamia armillata,* and *Badhamiopsis ainoae.,* Differences are outlined below.

—In *Badhamia affinis,* the capillitial tubes are filled with white lime and the spores are densely spinulose (Ing 1999, Martin & Alexopoulos 1969, Nannenga-Bremekamp 1991). These characters were not observed in our specimen.

—In *Badhamia armillata,* the capillitial tubes are also filled with white lime, but its spores are large (–15)17–18 (–19) µm with a distinct pale line and densely covered with dark wartlets (Nannenga-Bremekamp 1991).

—In *Badhamiopsis ainoae,* the capillitial tubes rise from base to peridium, and the spores are smaller (9–10 µm; Ing 1999, Martin & Alexopoulos 1969, Discover Life 2017).

Diderma deplanatum Fries, Syst. Myc. 3: 110 (1829) Pl. 5

Fructification short plasmodiocarps (curved or ring-shaped) or sessile sporangia, scattered, pulvinate, white or pale cream-colored, 1–1.5 mm diam. Hypothallus inconspicuous. Peridium double, the outer layer smooth, crustose, brittle, thick, white, the inner layer membranous, iridescent, orange below. Columella absent, represented by the thickened orange-brown base of the sporocarp. Capillitium made up of dark purple threads, which are simple or sparsely dichotomously branched, with a few and small dark bead-like thickenings. Spores in mass dark brown, yellow-brown in transmitted light, minutely spinulose, 9–11µm diam.

Specimen examined—**COMOROS ISLANDS, Ngazidja Island, Moroni city,** vegetable garden: 11°43.26′S 43°15.12′E, 34 m elev., on *Artocarpus heterophyllus* debris, 16.09.2016, Mohamed Seifou (MSY 160916-01c).

Comments—*Diderma deplanatum* has been found in Africa (Ndiritu & al. 2009, Wrigley de Basanta & al. 2013, Discover Life 2017). This species resembles *D. effusum,* which is separated by the presence of a distinct columella, at least when forming sporangia or short plasmodiocarps. *Diderma deplanatum* was at one time included in *D. niveum* as a lowland form, but *D. niveum* has a well-developed columella, is sporangiate, and is not flattened; only the orange colored inner layer is shared by both species (Ing 1999). *Diderma deplanatum* is distinguished from *D. platycarpum*

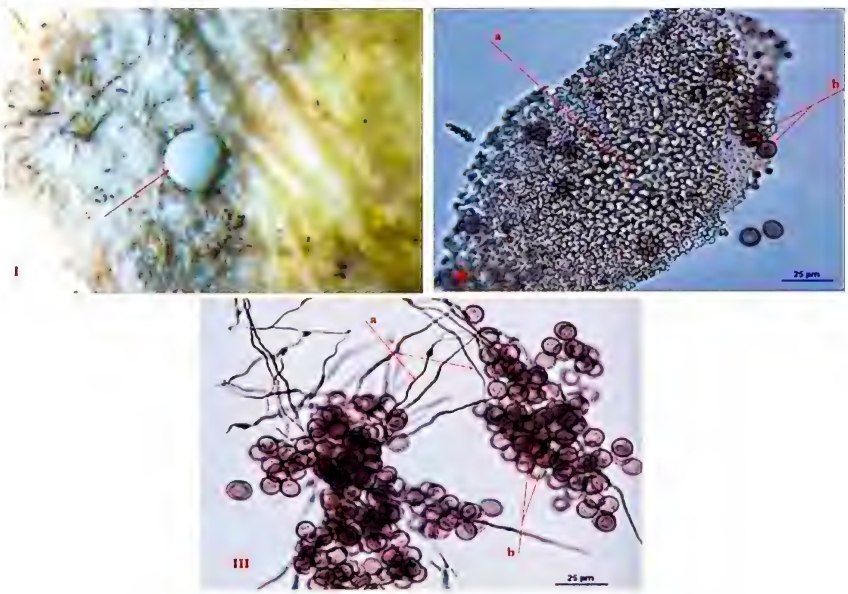

Plate 5. *Diderma deplanatum* (MSY 160916-01c).
I- individual sporocarp; II- a. peridium, b. spores; III- a. capillitial threads and spores.

by its less flattened plasmodiocarps that are simple or reticulate and not or only slightly plate-like, and its darker spores (Nannenga-Bremekamp 1991).

Didymium anellus Morgan, J. Cincinnati Soc. Nat. Hist. 16: 148 (1894) Pl. 6

Sporocarps plasmodiocarpic, perhaps broad or ring-shaped, sometimes sporangiate on a constricted base, infrequently short-stalked, varying to flat pulvinate, annulate, crustose, the surface pitted, white, or dingy gray to dark metallic. Hypothallus delicate, inconspicuous. Peridium single, membranous, purplish-brown, iridescent, covered with a rather sparse layer of lime crystals, dehiscence circumscissile. Columella absent or only suggested by an ochraceous or brownish deposit at the sporocarp base. Capillitium abundant, branched, and anastomosing slender dark threads that form an elastic net. Spores dark brown in mass, violet-brown in transmitted light, minutely warted (some warts found in clusters), 7–9 µm diam. Plasmodium transparent and colorless.

Specimen examined—**COMOROS ISLANDS, Ngazidja Island, Bibavou city,** vegetable garden: 11°34.58′S 43°17.15′E, 194 m elev., on *Carica papaya* litter materials, 16.09.2016, Mohamed Seifou (MSY160916-06).

Plate 6. *Didymium anellus* (MSY160916-06).
I- plasmodiocarps; II- mature plasmodiocarps covered with white lime crystals;
III- a. peridium, b. crystals, c. capillitium, d. spores.

Comments—*Didymium anellus* has been reported from Africa (Ndiritu & al. 2009, Wrigley de Basanta & al. 2013, Discover Life 2017).

Didymium minus (A. Lister) Morgan,
J. Cincinnati Soc. Nat. Hist. 16: 145 (1894) Pl. 7

Fructification, short-stalked sporangia, 0.8–1 mm tall, gregarious, depressed-globose or hemispherical, umbilicate below, white or pale gray, 0.4–0.6 mm diam. Hypothallus membranous, dark, usually forming disks under the sporangia. Stalk rather slender, faintly striate, partly submerged in the umbilicus, blackish or dark brown. Peridium membranous, delicate, covered with stellate lime crystals. Columella globose or depressed-globose,

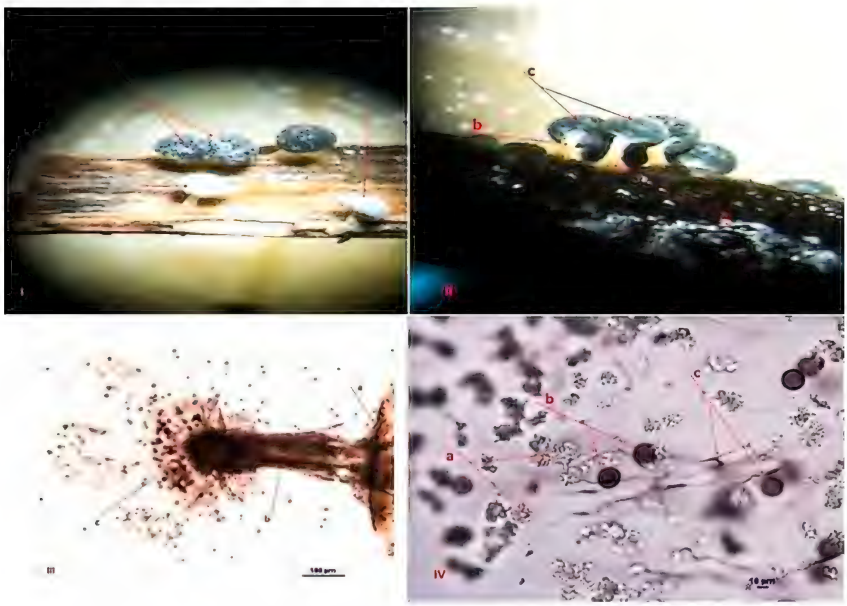

Plate 7. *Didymium minus* (MSY160916-02).
I- sporangium; II- a. hypothallus, b. stalk, c. peridium; III- a. hypothallus, b. stalk, c. columella; IV- a. crystals, b. capillitial threads, c. spores.

dark brown to sordid whitish, sometimes containing white crystalline lime. Capillitium delicate, colorless to lilac-grey, branching with few anastomoses. Spores dark brown in mass, dark violaceous brown by transmitted light, minutely warted, the warts often in groups, 8–12 µm diam. Plasmodium dark purplish-grey.

Specimen examined—**COMOROS ISLANDS, Ngazidja Island, Moroni city,** vegetable garden: 11°43.26′S 43°15.13′E, 36 m elev., on *Artocarpus altilis* litter material, 16.09.2016, Mohamed Seifou (MSY160916-02).

Comments—*Didymium minus* considered cosmopolitan (Ing 1999, Martin & Alexopoulos 1969, Lakhanpal & Mukerji 1981) and has been reported in Africa (Ndiritu & al. 2009, Discover Life 2017).

Didymium minus is separated from *D. melanospermum* by its smaller size and smaller paler spores and from *D. nigripes* by the short stalk (Ing 1999, Martin & Alexopoulos 1969, Nannenga-Bremekamp 1991).

Didymium nigripes (Link) Fries, Syst. Myc. 3: 119 (1829) Pl. 8

Fructifications sporangiate with a long stalk <1.5 mm long, globose or subglobose, umbilicate below, white or ashy-grey, 0.3–0.5 mm diam. Hypothallus

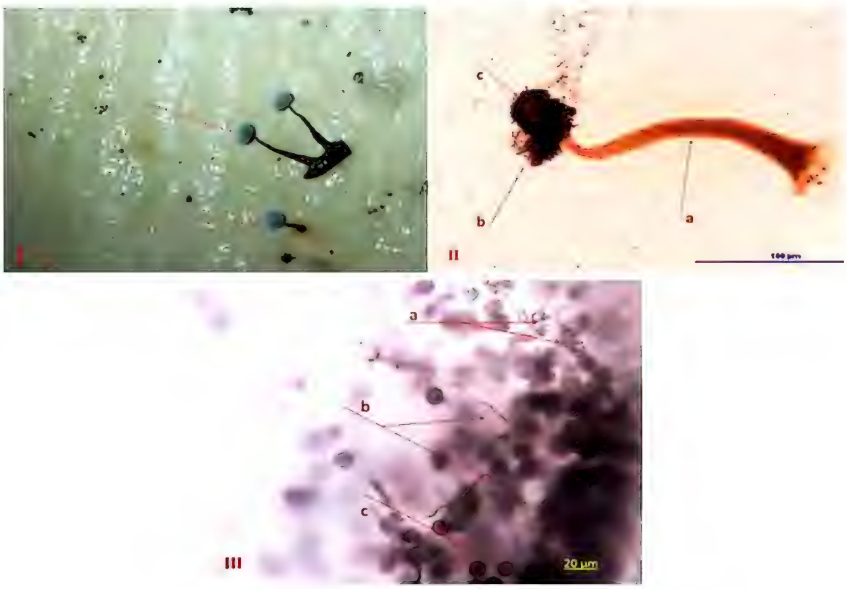

Plate 8. *Didymium nigripes*. (MSY 160916-07).
I- sporangium; II- a. stalk, b. spore mass, c. columella;
III- a. peridial crystals, b. capillitial threads, c. spores.

discoid, dark or blackish. Stalk slender, dark brown to blackish, slightly paler or red-brown towards the top, translucent red-brown in transmitted light, often filled with dark amorphous matter below. Peridium single, membranous, grey-brown, covered with white stellate lime crystals. Columella subglobose, dark brown, calcareous. Capillitium delicate, pale brown or colorless, sparsely dichotomously branched. Spores dark brown in mass, pale violaceous brown in transmitted light, minutely warted and the warts arranged in clusters, 7–9 µm diam. Plasmodium brown-grey or colourless

Specimen examined—**COMOROS ISLANDS, Ngazidja Island, Bibavou city,** vegetable garden: 11°34.594′S 43°17.154′E, 195 m elev., on *Pterocarpus indicus* debris, 16.09.2016, Mohamed Seifou (MSY 160916-07).

Comments—*Didymium nigripes* is regarded as cosmopolitan (Ing 1999, Martin & Alexopoulos 1969, Lakhanpal & Mukerji 1981) and has been previously recorded from Africa (Ndiritu & al. 2009, Wrigley de Basanta & al. 2013, Discover Life 2017).

Didymium nigripes can be distinguished from *D. minus* by its thinner, longer, and mostly translucent stalk (Nannenga-Bremekamp 1991) and from *D. iridis* by its mottled peridium (Ing 1999).

Didymium serpula Fries, Syst. Myc. 3: 126 (1829) Pl. 9

Fructifications plasmodiocarps, solitary or in small groups, sessile, effused, perforated or nearly continuous, 0.1–0.15 mm thick, 2–8 mm long (sometimes much longer, ≤4 cm), dark grey or greyish-white. Hypothallus inconspicuous. Peridium membranous, dark grey or iridescent, sparsely to densely covered with stellate or irregular white lime crystals. Columella absent. Capillitium thin, dichotomously branched, with few or many anastomoses, yellow-brown or pale threads attached to a subglobose vesicle 30–50 µm diam. filled with yellow granular material. Spores brown in mass, pale violaceous brown or pale brown in transmitted light, covered with very fine warts, 7–9 µm diam.

Specimen examined—**COMOROS ISLANDS, Ngazidja Island, Moroni city,** vegetable garden: 11°43.26′S 43°15.12′E, 34 m elev., on *Artocarpus heterophyllus* debris, 16.09.2016, Mohamed Seifou (MSY 160916).

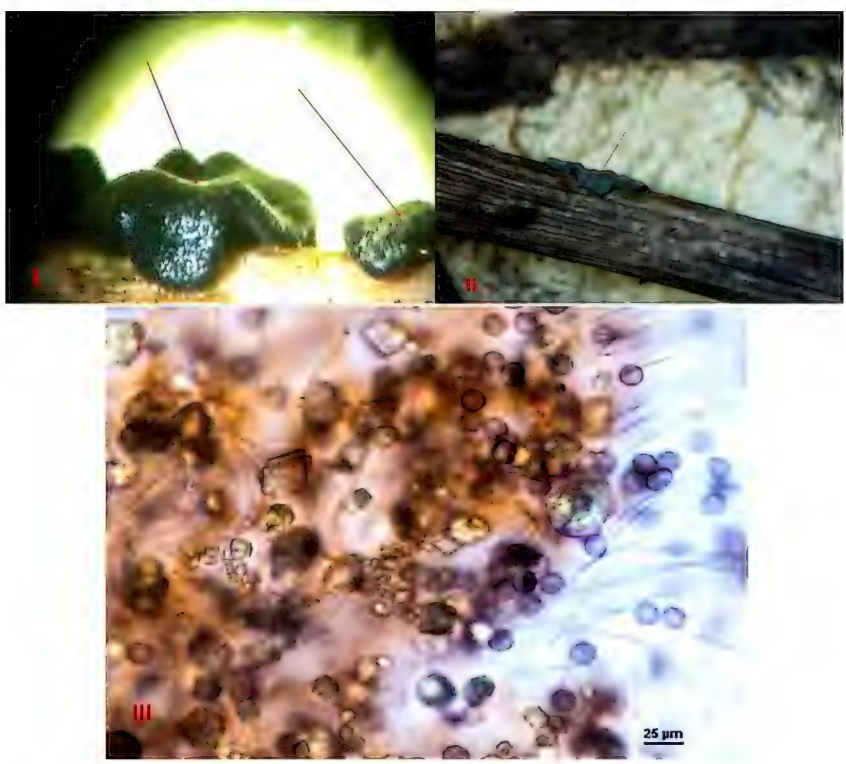

Plate 9. *Didymium serpula* (MSY 160916).
I- plasmodiocarps; II- plasmodiocarp covered with lime;
III- crystals (stellate or irregular), capillitial threads and spores.

Comments—*Didymium serpula* has been reported from Africa (Ndiritu & al. 2009, Ing 1999, Discover Life 2017).

Didymium serpula is distinguished from *D. flexuosum* Yamashiro, which produces capillitium that are not attached to the vesicle and differently shaped fructifications (Nannenga-Bremekamp 1991).

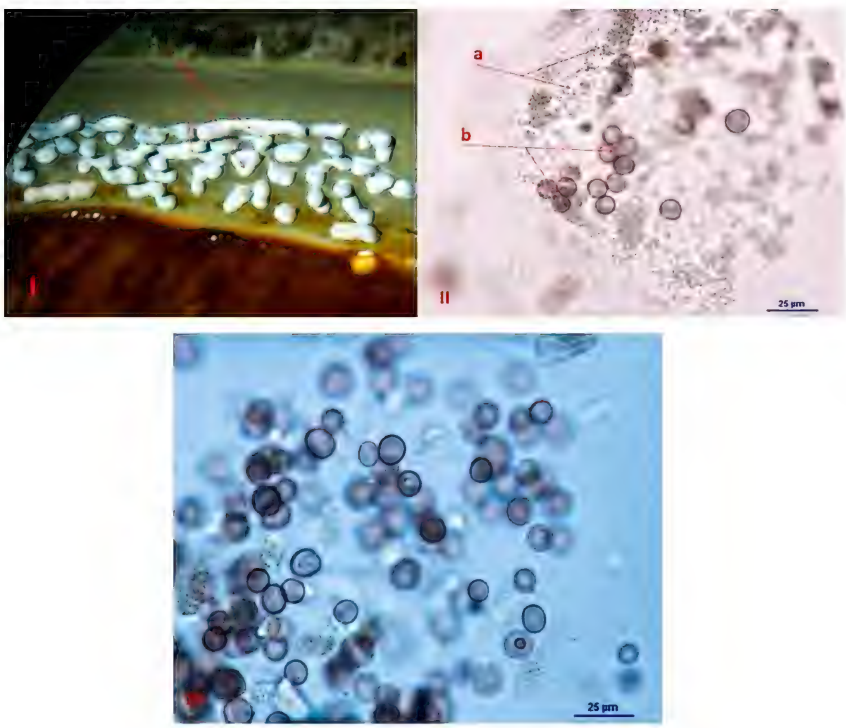

Plate 10. *Physarum cinereum* (MSY 160917-05a).
I- plasmodiocarps; II- a. peridium, b. spores; III- capillitium and spores.

Physarum cinereum (Batsch) Pers., Neues Mag. Bot. 1: 89 (1794) Pl. 10

Fructifications sessile sporangiate, subglobose or short to elongated plasmodiocarps, closely gregarious, crowded or heaped, 0.3–0.5 mm broad, ash-grey or white (occasionally iridescent from lack of lime). Hypothallus inconspicuous and colourless. Peridium single, membranous, colorless or pale brown, encrusted with a more-or-less white lime deposit. Columella absent. Capillitium abundant, with spherical or angular large white nodes, hyaline or with calcareous deposit, sometimes almost badhamioid. Spores purplish-brown in mass, clear violaceous in transmitted light, minutely

warted, 9–13 µm diam. Plasmodium watery-white or colorless, often becoming bright

Specimen examined—**COMOROS ISLANDS, Ngazidja Island, Ntsorale city,** edge of the road: 11°41.227′S 43°24.768′E, 243 m elev., on *Ricinus communis* aerial and litter materials, 17.09.2016, Mohamed Seifou (MSY 160917-05a).

Comments—*Physarum cinereum* is regarded as cosmopolitan (Martin & Alexopoulos 1969, Ing 1999, Lakhanpal & Mukerji 1981) and has been reported from Africa (Ndiritu & al. 2009, Wrigley de Basanta & al. 2013, Discover Life 2017).

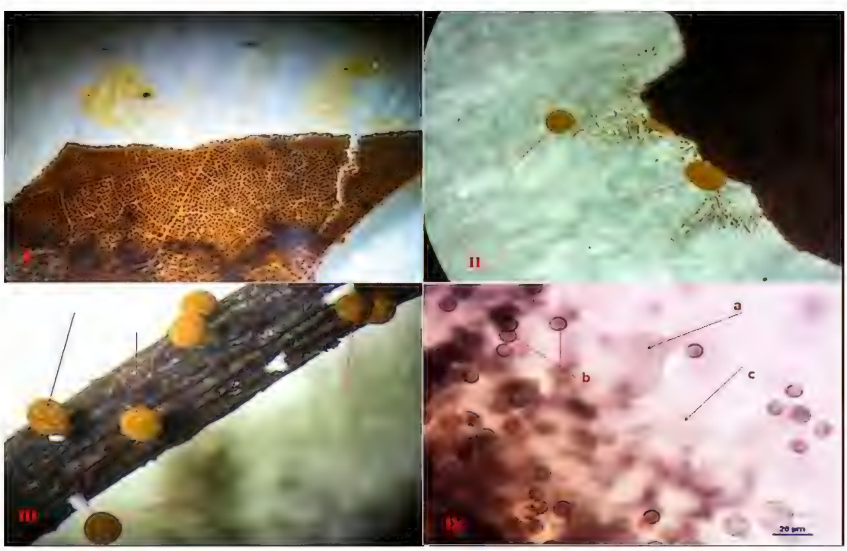

Plate 11. *Physarum melleum* (MSY 160917-03).
I- plasmodium; II- sclerotium; III- sporangia; IV- capillitium and spores.

Physarum melleum (Berk. & Broome) Massee,
Monogr. Myxogastr.: 278 (1892) Pl. 11

Fructifications sporangiate, gregarious, stipitate, rarely sessile, mostly globose, 0.4–0.5 mm diam., frequently yellow to dull orange, but varying from yellowish grey or honey yellow to bright orange-red or reddish brown. Hypothallus white or colorless. Stalk small (≤50% of the total height of the sporangium), cylindrical or tapering upward, stout, opaque, white, sometimes ochraceous or tawny, furrowed, calcareous. Peridium single, rugose, persistent at the base, encrusted with lime. Columella small, calcareous, conical, color white, yellowish, pallid, rarely orange. Capillitium abundant, the nodes large, angular, white (less commonly yellow or orange),

internodes small and delicate. Spores globose, dark brown in mass, pale violet-brown in transmitted light, minutely warted, 7.5–9 µm diam. Plasmodium yellow or olive-green.

Specimen examined—**COMOROS ISLANDS, Ngazidja Island, M'vouni city,** vegetable garden: 11°43.27′S, 43°16.54′E, 395 m elev., on *Litchi sinensis* aerial and litter materials, 17.09.2016, Mohamed Seifou (MSY160917-03).

Comments—*Physarum melleum* is regarded as cosmopolitan (Lakhanpal & Mukerji 1981, Martin & Alexopoulos 1969) and has been reported previously from Africa (Ndiritu et al 2009, Wrigley de Basanta & al. 2013, Discover Life 2017).

Physarum notabile T. Macbr., N. Amer. Slime-Moulds ed. 2: 80 (1922) Pl. 12

Fructification sporangiate, stalked, occasionally sessile, up to 1,5 mm in length or forming short plasmodiocarps, gregarious, globose to kidney-shaped,

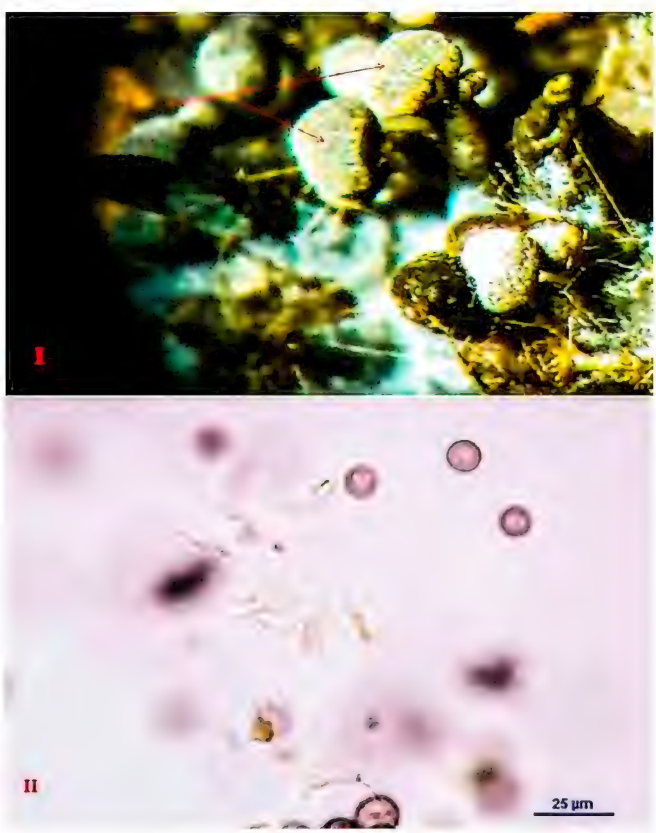

Plate 12. *Physarum notabile* (MSY 160917-05b).
I- sporangium; II- capillitium and spores.

white, 0.3–1 mm diam. Hypothallus concolorous with the stalk. Stalk (when present) short, weak to stout, tapering upward, plicate-furrowed, opaque, dark or covered with white calcareous granules. Peridium single, membranous, densely covered with ashy white calcareous deposit, the basal peridium persisting as a broad cup. Columella absent. Capillitium abundant, nodes variable in size and shape, connected by long hyaline threads. Spores black in mass, olivaceous brown in transmitted light, minutely warted, 9–12 µm diam.

Specimen examined—**COMOROS ISLANDS, Ngazidja Island, Ntsorale city,** edge of the road: 11°41.23′S 43°24.77′E, 243 m elev., on *Ricinus communis* aerial and litter materials, 17.09.2016, Mohamed Seifou (MSY 160917-05b).

Comments—*Physarum notabile* has been collected previously in Africa (Ndiritu & al. 2009, Wrigley de Basanta & al. 2013, Discover Life 2017).

The sessile or plasmodiocarpous forms may be mistaken for forms of *P. compressum* with much darker spores, so it is very important to check the spore colour carefully (Ing 1999).

Acknowledgments

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Soleella mirabilis sp. nov. on *Saccharum arundinaceum* from China

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Abstract—A new species, *Soleella mirabilis*, on leaves of *Saccharum arundinaceum* from China, differs from all other species of *Soleella* mainly by its consistently trifusiform ascospores deeply constricted at the isthmuses. A technical description, comments, and illustrations of this fungus are provided. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words—morphology, *Poaceae, Rhytismataceae*, *Rhytismatales,* taxonomy

Introduction

The genus *Saccharum* L., which includes some of the most important crop plants in the world, has been extensively studied for its associated fungi. There are, however, very few records of members of the *Rhytismatales* associated with these plants. Only three species have been reported: *Terriera sacchari* (Lyon) P.R. Johnst., *Lophodermium arundinaceum* (Schrad.) Chevall., and *L. saccharicola* S.J. Wang & Y.R. Lin (Johnston 2001, Lin & al. 2012, Wang & al. 2012, Farr & Rossman 2018). In the present paper, we describe a new

[A] Shi-Juan Wang and Xing-Yan Zhao contributed equally to this work.

species of the *Rhytismataceae* on dead leaves of *Saccharum arundinaceum* from Anhui and Jiangxi Provinces, China.

Materials & methods

Mature fruit bodies were selected from collected specimens. Macroscopic observations were made of dried material using a dissecting microscope at 10–50× magnification. The material was then rehydrated in water for c. 15 min., and 8–15 μm thick vertical sections of the fruit bodies were sliced using a freezing microtome or razor blades by hand. Sections were mounted in water or 0.1% (w/v) cotton blue in water for the observations of ascomatal and conidiomatal outlines in vertical section. Gelatinous sheaths surrounding ascospores and paraphyses were examined in water. The colours of internal structures and ascospore contents were observed in water. Measurements and drawings were made using materials mounted in water from c. 30 asci, ascospores, and paraphyses per specimen. Point and line integrated illustration of internal structures of fruit bodies were drawn using the microscopic painting device. The holotype specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Taxonomy

Soleella mirabilis S.J. Wang & Y.R. Lin, **sp. nov.** Figs 1, 2

MycoBank MB 825184

Differs from *Soleella chinensis*, *S. huangshanensis*, and *S. pinicola* by its occurrence on a poaceous host, its subepidermal ascomata, and its consistently trifusiform ascospores deeply constricted at the isthmuses.

Type: On dead leaves of *Saccharum arundinaceum* Retz. (*Poaceae*): China, Anhui, Huangshan Arboretum, alt. c. 550 m, 25 September 2004, S.J. Wang & Y.R. Lin 1924b (**Holotype**, AAUF 68032b).

Etymology: The specific epithet refers to the extraordinary shape of the ascospores.

Colonies on both sides of leaves, forming irregular grey-yellow to pale brown bleached spots that sometimes coalesce into larger bleached spots.

Zone lines frequent, grey-brown or black, thin, entirely or partly surrounding the bleached spots.

Conidiomata developing on both sides of leaves, scattered, occasionally several coalescent. In surface view, conidiomata 140–270 × 70–130 μm, oblong, rounded or slightly irregular, brown to black-brown, opening by one to several ostioles. In vertical section conidiomata intraepidermal, more or less double lens-shaped. Upper wall and basal wall poorly developed. Conidia not observed.

Ascomata in similar positions to conidiomata on the host, mostly parallel to the host veins, scattered, rarely 2–3 ascomata confluent, in pale brown

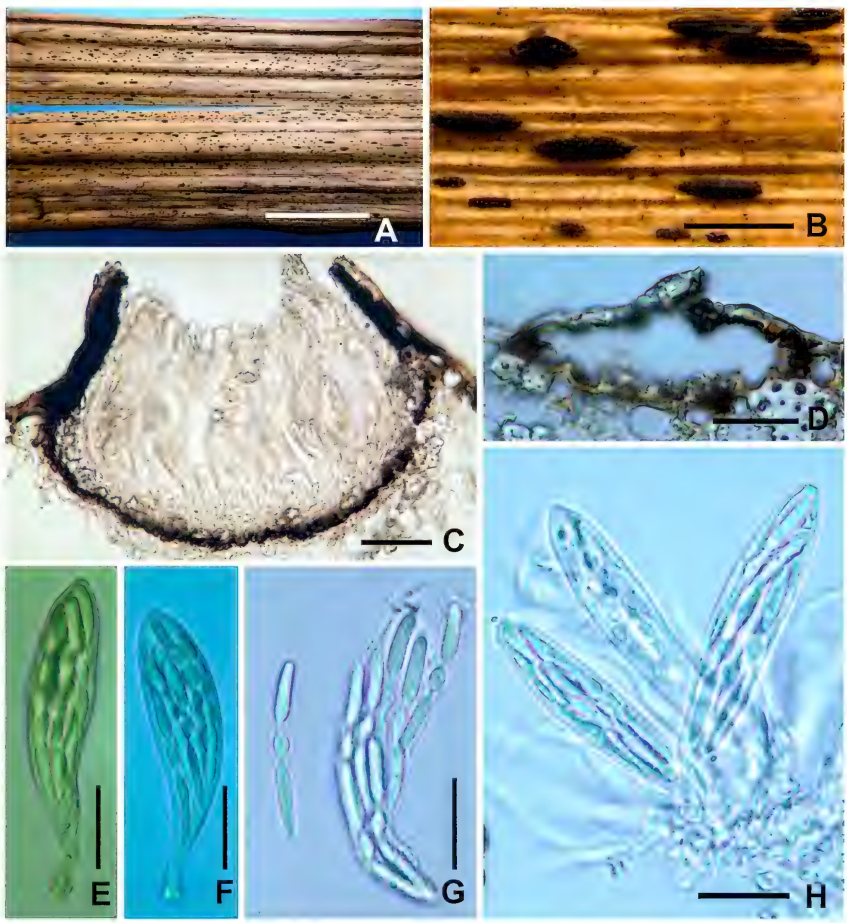

Fig. 1. *Soleella mirabilis* (holotype, AAUF 68032b) on *Saccharum arundinaceum*: A. Habit on a leaf; B. Conidiomata and ascomata observed under a dissecting microscope; C. Ascoma in median vertical section; D. Conidioma in median vertical section; E, F. Asci; G. Ascospores; H. Asci and paraphyses. Scale bars: A = 10 mm; B = 1 mm; C = 50 µm; D = 30 µm; E– H = 20 µm.

bleached areas and associated with zone lines. In surface view, ascomata 570–1150 × 180–250 µm, elliptical, long elliptical, or oblong-elliptical, grey-black or dark brown, sometimes with a light surrounding area and a dark brown perimeter line, shiny, ends acute, round or obtuse, moderately rising above the surface of the substratum and opening by a single longitudinal split running the entire length of the ascoma. Lips absent, but with pale preformed opening

mechanism. In median vertical section, ascomata subepidermal. Covering stroma 18–25 µm thick near the centre of the ascoma, extending to the basal stroma, consisting of an outer layer of host cuticle and an inner layer of textura angularis with dark brown, thick-walled cells 3–7 µm diam. Lips cells absent, but some thin-walled, hyaline, small angular cells visible near the opening. Basal stroma poorly developed, slightly concave to flat, 3–11(–18) µm thick, composed of 1–2 rows of dark brown, thick-walled angular cells 5–9 µm diam. Subhymenium 16–23 µm thick, textura porrecta to angularis. Paraphyses 10–25 µm longer than asci, filiform, septate, not branched, not (or only slightly) swollen or slightly swollen at the apex, with a c. 1 µm thick gelatinous sheath. Asci ripening sequentially, (60–)75–110 × (13–)15–19 µm, broad clavate to saccate, stalk 10–25 µm long, thin-walled, apex rounded or rarely subtruncate, J–, without circumapical thickening, 8-spored, discharging spores though a single apical pore. Ascospores (32–)38–42 × (2–)3–4.5 µm, consistently trifusiform, upper part cylindrical, middle part elliptical or sub-rounded and lower part slightly clavate, rounded at the apex and subacute or rounded at the base, hyaline, aseptate, deeply constricted at the isthmuses, in the thinnest part <1 µm diam, with a 1–1.5 µm thick gelatinous sheath.

Host species, habitat, distribution: On *Saccharum arundinaceum*, producing conidiomata and ascomata on dead leaves. Known from Anhui and Jiangxi Provinces, China.

Additional specimens examined: On *Saccharum arundinaceum*: **China**, **Anhui**, **Tiantangzhai**, alt. c. 900 m, 17 Sep. 2005, Y.R. Lin & S.J. Wang 2025 (AAUF 68133); **Shitai County**, Dayan, alt. c. 200 m, 1 Jul. 2006, S.J. Wang & T. Zhang 2092 (AAUF 68200); **Huangshan Arboretum**, alt. c. 550 m, 11 Sep. 2007, Y.R. Lin, S.J. Wang & L. Chen 2232a (AAUF 68340a); **Wanfoshan**, alt. c. 950 m, 22 Aug. 2008, Y.R. Lin & S.J. Wang 2343 (AAUF 68451); **Jiangxi**, **Mount Sanqingshan National Park**, Daye Forest Farm, alt. c. 600 m, 20 Aug. 2012, Y.R. Lin, S.J. Wang & F. Zhou 2650 (AAUF 68758).

Comments—Within *Soleella*, *S. mirabilis* is exceptional because of its consistently trifusiform ascospores deeply constricted at the isthmuses. Within *Rhytismatales*, some species of *Bifusella* and *Soleella* have similarly shaped ascospores (Darker 1967, Lin 1994, Lin & al. 2012). *Bifusella* species have subcuticular ascomata, and *Soleella* species have intraepidermal to partly subhypodermal ascomata; we therefore place our new species in *Soleella*. *Soleella* was established by Darker (1967) and includes six currently accepted species (Index Fungorum 2018), all exclusively on conifer needles and including important plant pathogens, e.g., *S. chinensis* causing serious needle-cast or dieback (Darker 1967, Lin & al. 2012). However, our new species, *S. mirabilis*, has a poaceous host.

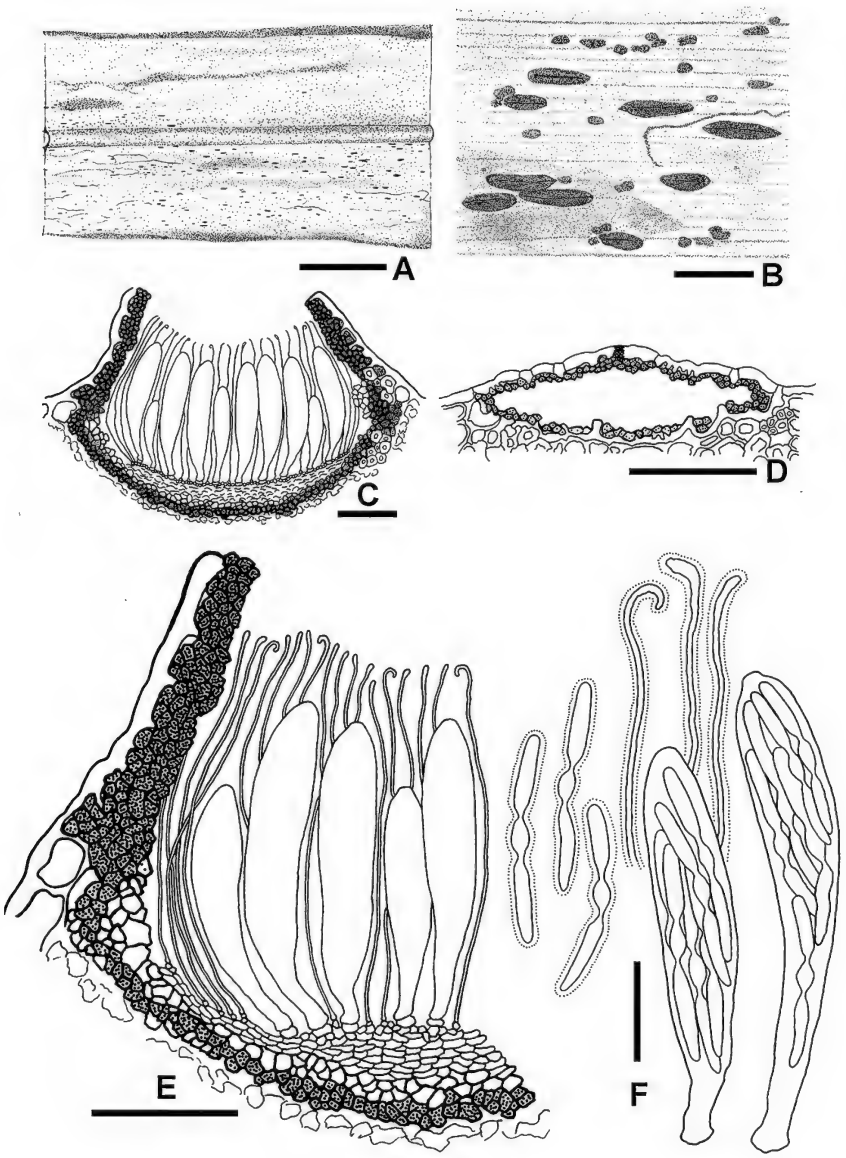

Fig. 2. *Soleella mirabilis* (holotype, AAUF 68032b) on *Saccharum arundinaceum*: A. Habit on a leaf; B. Conidiomata, ascomata, and a zone line observed under a dissecting microscope; C. Ascoma in median vertical section; D. Conidioma in median vertical section; E. Portion of ascoma in median vertical section; F. Paraphyses, asci and ascospores. Scale bars: A = 10 mm; B = 1 mm; C– E = 50 μm; F = 20 μm.

Soleella chinensis Y.R. Lin & al., *S. huangshanensis* C.L. Hou & H.S. Cao, and *S. pinicola* Y.R. Lin & W. Ren also produce trifusiform ascospores, but only rarely; they produce mostly bifusiform ascospores that are only slightly constricted at the isthmuses (Lin & Ren 1992; Lin & al. 1995, 2012, Hou & al. 1997).

Our new species is distributed widely throughout central and eastern China (Jiangxi and Anhui Provinces). Sizes of ascomata, asci, and ascospores varied among different collections, being larger in specimens on flourishing host plants, and smaller on thin and weak hosts. We infer that nutritional status of substrate influences the development of the specimens. We tried unsuccessfully to extract genomic DNA for further analysis.

Acknowledgments

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Brachyconidiella monilispora, a rare fungus newly recorded from South America

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Abstract—*Brachyconidiella monilispora* was collected during an inventory of conidial fungi associated with decaying leaves of *Hancornia speciosa* in sandbank areas in Bahia state, Brazil. This rare fungus, previously recorded only from Cuba and Florida (U.S.A.), is a new record for South America. The Brazilian material is described and illustrated.

Key words—asexual fungi, hyphomycete, tropical fungi, taxonomy

Introduction

Brachyconidiella is a monotypic genus for a type species *B. monilispora* described from decaying leaves of *Coccoloba uvifera* L. (*Polygonaceae*) in Cabo de San Antonio, Pinar del Río, Cuba (Castañeda-Ruiz & Kendrick 1990). The species was recollected twice in Cuba (1995, 1996) from unidentified rotten leaves and isolated in pure culture (MUCL39848, MUCL39860). Subsequently, the species was found in Key Largo, Florida, U.S.A., from dead leaves and stems of an unidentified plant (Delgado 2009). *Brachyconidiella monilispora* is morphologically similar to species in another monotypic genus, *Brachyconidiellopsis fimicola* Decock & al. (Decock & al. 2004). Both species

† In memoriam

have cheirosporous, penicilliform, repeatedly branched, moniliform conidia that develop from holoblastic conidiogenous cells and are associated with sporodochial or synnematal conidiomata (Castañeda-Ruiz & Kendrick 1990, Decock & al. 2004, Seifert & al. 2011). However, the genera differ in their conidiogenous cell features and conidial secession: *Brachyconidiellopsis* has monoblastic (or commonly polyblastic) conidiogenous cells and rhexolytic conidial secession whereas *Brachyconidiella* has monoblastic conidiogenous cells and schizolytic conidial secession. Moreover, these two genera have different habits, *Brachyconidiellopsis* in herbivore feces (coprophilous?) and *Brachyconidiella* in decomposing plant litter. Phylogenetic analyses place *Brachyconidiella* as incertae sedis within *Dothideomycetes*, and *Brachyconidiellopsis* within *Microascaceae*, *Microascales*, *Sordariomycetes* (Decock & al. 2004, Seifert & al. 2011).

Materials & method

Samples of decaying leaves of *Hancornia speciosa* occurring in sandbank areas were collected during expeditions to the Conde, Bahia, Brazil. The samples were placed in paper bags and sent to the laboratory to undergo washing processes (Castañeda-Ruiz & al. 2016). Thereafter, the samples were observed under stereomicroscope, and the microscopic reproductive structures were transferred to slides containing PVL resin (polyvinyl alcohol, lactic acid and phenol). The specimens analyzed were deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Brachyconidiella monilispora R.F. Castañeda & W.B. Kendr., Univ. Waterloo Biol. Ser. 33: 14, 1990. Fig. 1

Mycelium mostly immersed in the substrate, composed of septate, branched smooth walled, brown hyphae, 1–2 µm. Conidiomata synnematal, scattered, erect, straight or somewhat flexuous, brown at the base to pale brown towards apex, 26–55 × 6–10 µm, head 35–53 µm diam. No sporodochial conidiomata were observed. Conidiophores unbranched or rarely branched, 1–3-septate, smooth, brown to pale brown at apex, 2–2.5 µm wide. Conidiogenous cells monoblastic or polyblastic, terminal, integrated, barrel-shaped, pale brown, 4–6 × 1.5–2 µm. Conidial secession schizolytic. Conidia 15–19 × 14–27 µm, cheirosporous, penicilliform, moniliform, branched (7–13) with chains composed of 3–8 cells and 12.5–16 µm long; lower cells of branches more or less spherical, 2 µm wide, basal cells truncated, somewhat trapezoid, 3 µm wide, subhyaline.

Fig. 1. *Brachyconidiella monilispora* (HUEFS 237347).
A–C. Conidia; D, E. Detail of conidiogenous cells; F. General aspect of conidioma.
Scale bars: A–E = 5 μm; F = 10 μm.

Specimen examined: **BRAZIL**, **Bahia State**, **Conde**, Sítio do Conde, 11°25′S 37°36′W, on dead leaves of *Hancornia speciosa* Gomes (*Apocynaceae*), 10 August 2017, coll. D.R.C. Silva (HUEFS 237347).

Note: The features of the Brazilian specimen are in accord with the earlier descriptions, except for the smaller conidiomata (Cuban specimens 70–120 ×

11–18 µm; Floridian specimen <190 × 11–44 µm) and the presence of some polyblastic conidiogenous cells. The Brazilian and Floridian specimens both lacked sporodochial conidiomata; and the Brazilian and Cuban specimens both produced branched conidiophores. This is the first record of *B. monilispora* from South America. Unfortunately, the fungus does not develop in pure culture.

In addition to *Brachyconidiellopsis*, two other genera have similar conidiation to *Brachyconidiella monilispora*. *Matsushimaea* Subram. (particularly *M. fasciculata* Subram.) also has monoblastic conidiogenous cells and cheirosporous, repeatedly branched, moniliform conidia with schizolytic secession but differs by its brown conidia and the absence of conidiophores (Subramanian 1978, Seifert & al. 2011). According to Decock & al. (2004), some asexual species of *Seuratia* Pat. (particularly *S. millardetii* (Racib.) Meeker) have similar branched conidia composed of moniloid chains of spheroid cells but differ by their sporochial conidiomata (Meeker 1975). No sequences were available for *Matsushimaea* and *Seuratia* for phylogenetical comparison with *B. monilispora*.

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Conidiobolus antarcticus, a synonym of *C. osmodes*

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Abstract—DNA from two ribosomal regions (nrLSU and mtSSU) and one protein coding region (RPB2) was sequenced and analysed for ten *Conidiobolus* isolates, including the ex-type isolates of *C. antarcticus* and *C. osmodes*, and a detailed morphological comparison of these two species was compiled. *Conidiobolus antarcticus* formed a highly supported (100%) clade with three *C. osmodes* isolates in the phylogenetic tree based on the combined data sets of these three genes. The two species were similar in the colony diameter, hyphal diameter, primary conidial size and shape, and zygospore formation, size, and shape. Therefore, we consider *C. antarcticus* to be a synonym of *C. osmodes*.

Key words—*Ancylistaceae, Entomophthorales,* taxonomy

Introduction

Conidiobolus antarcticus, isolated from the Antarctic mosses *Scistidium antarctici* and *Hennediella heimii*, was first described by Tosi & al. (2004). In size and shape of the primary conidia and zygospores, *C. antarcticus* resembles *C. osmodes* (Drechsler 1954), isolated from decaying plant detritus in Louisiana, U.S.A. When we carried out a study on the phylogenetic systematics of *Conidiobolus*, we included the ex-type cultures of *C. antarcticus* and *C. osmodes*. A three-gene molecular analysis and morphological comparisons indicated that these two species are conspecific.

Materials & methods

Fungal isolates and culturing conditions

The isolates were provided by the USDA-ARS Entomopathogenic Fungus Collection, Ithaca, New York, U.S.A. (ARSEF) and the Research Center of Entomopathogenic

Table 1. *Conidiobolus* and *Basidiobolus* species and strains used in this study, with GenBank accession numbers.

Species	Strain	nrLSU	RPB2	mtSSU
C. antarcticus	ARSEF 6913 [T]	DQ364207	DQ364217	DQ364227
C. bangalorensis	ARSEF 449 [T]	DQ364204	DQ364214	DQ364225
C. coronatus	ARSEF 206	DQ364201	DQ364211	DQ364221
	ARSEF 421	DQ364203	DQ364213	DQ364223
	ARSEF 525	DQ364205	DQ364215	DQ364224
C. lamprauges	ARSEF 2338	DQ364206	DQ364216	DQ36422
C. osmodes	RCEF 4447	JN131539	MH401096	MH401095
	ARSEF 79	DQ364199	DQ364209	DQ364219
	ARSEF 193 [T]	DQ364200	DQ364210	DQ364220
C. thromboides	ARSEF 70	DQ364198	DQ364208	DQ364218
B. microsporus	ARSEF 265	DQ364202	DQ364212	DQ364222

[T], ex-type culture

Fungus, Hefei, Anhui, China (RCEF). The species, strains, and GenBank sequence accession numbers are given in Table 1.

The mycelium was transferred to a Petri dish containing a Sabouraud dextrose agar yeast extract culture medium (SDAY; 4% dextrose, 1% peptone, 1% yeast extract, 1.5% agar) and covered with autoclaved cellophane. The Petri dish was sealed with parafilm and placed in an incubator at 25°C for approximately 1–2 weeks or until the fungus had grown to the edge of the cellophane. The mycelia were scraped from the cellophane and stored at –20°C until DNA extraction.

DNA extraction, amplification, and sequencing

Mycelia were ground in a sterile 1.5 mL microcentrifuge tube with a small plastic stick. The extraction was performed using benzyl chloride according to the method of Zhu & al. (1994). All DNA samples were then diluted 1:20 for PCR reactions and stored at –10°C.

Regions of three genes were amplified by PCR: nuclear ribosomal large subunit (nrLSU) by primers LR0R and LR5 (Vilgalys & Hester 1990), mitochondrial small subunit of rDNA (mtSSU) by MtSSUc-2F and MtSSUc-2R (http://aftol.biology.duke.edu/pub/primers/getPrimerDetails?primer_id=258), and RNA polymerase unit II second large subunit (RPB2) by fRPB2-7F and RPB2-11aR (Liu & al. 1999). All procedures used in this study for LSU amplification have been described previously (Liu & al. 2005).

The PCR reaction mixture for mtSSU contained 200 µM each dNTP, 1X Mg-free buffer, 2.5 mM $MgCl_2$, 0.5 µM each primer, 3% DMSO, 10–50 ng genomic DNA and 0.04 Unit/ µL Taq polymerase (Promega). The PCR program comprised an initial

denaturation step of 95°C for 5 min, 34 cycles of 94°C for 1 min, 54°C for 2 min, 72°C for 2 min, and a final extension at 72°C for 10 min.

The PCR reaction mixture for RPB2 was the same as the mtSSU mixture, but without DMSO. A hot start PCR technique was performed, and the PCR conditions were 100°C for 5 min, an initial denaturation step 95°C for 5 min (during which Taq polymerase was added to each tube), 34 cycles of 94°C for 1 min, 49.2°C for 2 min, 72°C for 2 min, and a final extension at 72°C for 10 min.

PCR products were purified using the QIAquick PCR purification kit® and sequenced in both directions using the initial amplification primers at the Biotechnology Resource Center at Cornell University.

Phylogenetic analysis

The sequences were aligned using Clustal 1.83 (Thompson & al. 1994), and the alignment was refined by eye. *Basidiobolus microsporus* R.K. Benj. was used as the outgroup species. Parsimony analysis was performed in PAUP* version b10 (Swofford 2002) using a heuristic search. A starting tree was obtained via a 'closest' addition sequence, tree-bisection-reconnection was used as the branch-swapping algorithm, and MULTREES was switched off. Character states were unordered, and characters were equally weighted. Bootstrap values were calculated based on 1000 replicates of a heuristic fast stepwise addition search.

Bayesian analysis was carried out using MrBayes 3.0b4 (Huelsenbeck 2000, Huelsenbeck & al. 2001). We used a six-parameter model to run four chains for 500,000 generations, saving a tree every 100 generations. The first 500 trees were discarded (burn in), and the remaining trees were saved to a file. A 50% majority rule consensus tree was then calculated using PAUP*. Tree topology matches the combined parsimony analysis.

Results & discussion

nrLSU phylogeny

PLATE 1A

Parsimony analysis of the nrLSU data set yielded one Maximum Parsimony tree (MPT) of 865 steps with CI = 0.884 and RI = 0.902. *Conidiobolus antarcticus* formed an unresolved clade with the three *C. osmodes* isolates in the strict consensus. There was 99% identity between *C. antarcticus* and *C. osmodes* ARSEF79 (923/925 due to two point indels, both in *C. antarcticus*) according to a Pairwise Blast. Among the three isolates of *C. osmodes*, the gene sequences differed by 0–1 base positions.

mtSSU phylogeny

PLATE 1B

Parsimony analysis of the mtSSU data set yielded one MPT of 177 steps with CI = 0.927 and RI = 0.940. *Conidiobolus antarcticus* formed a clade with the three *C. osmodes* isolates. There was 100% identity among the isolates of *C. osmodes* and *C. antarcticus*.

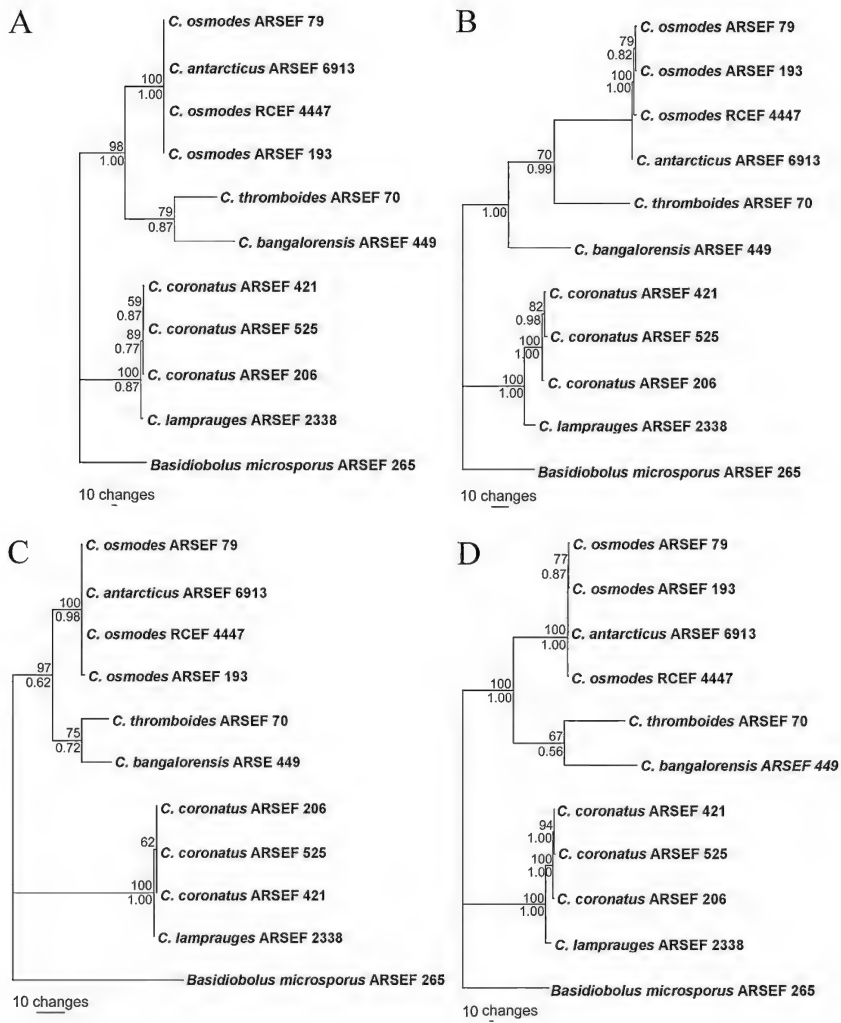

Plate 1. Phylogenetic relationships among ten strains from six *Conidiobolus* species, based on the data set from different genes, with *Basidiobolus microsporus* as outgroup. Bootstrap proportions (BP) and Bayesian posterior probabilities (PP) >50% are indicated above the branches. A. Tree based on the nrLSU gene. B. Tree based on the mtSSU gene. C. Tree based on the RPB2 gene. D. Tree based on the combined data set of these three genes.

RPB2 phylogeny PLATE 1C

Parsimony analysis of the RPB2 data set yielded one MPT of 621 steps with CI = 0.773 and RI = 0.820. *Conidiobolus antarcticus* formed an unresolved clade with the three *C. osmodes* isolates in the strict consensus. The relative positions of *C. bangalorensis* and *C. thromboides* differed from the other two gene trees, but the trees were not in significant conflict. There was 99% identity between *C. antarcticus* and *C. osmodes* ARSEF79 (869/875 due to mismatches: C:N, T:C, A:C, T:N, T:C, –:A) according to a Pairwise Blast. Among the three isolates of *C. osmodes*, the gene sequences differed by 0–6 base positions, and the three *C. osmodes* isolates sequences were 99% identical.

Combined LSU, mtSSU and RPB2 phylogeny PLATE 1D

One MPT of 1647 steps with CI = 0.856 and RI = 0.882 was obtained from the parsimony analysis of the combined data set. *Conidiobolus antarcticus* formed a short clade with the three *C. osmodes* isolates with 100% bootstrap support. Bayesian and parsimony analyses both gave no resolution within the *C. osmodes* and *C. antarcticus* clades.

Conidiobolus antarcticus was separated from *C. osmodes* type isolates by a few substitutions and indels, but these interspecies differences in nucleotide sequence fell within the range of infrageneric nucleotide divergence among the three *C. osmodes* isolates. The three *C. coronatus* isolates showed similarly wide infraspecific differences: LSU sequences differed by 0–2 base positions; mtSSU sequences did not differ; and PRB2 sequences differed by 5–10 base positions.

The analyses of phylogenetic tree and nucleotide divergence support *Conidiobolus antarcticus* and *C. osmodes* as the same species at the DNA level.

Morphological analysis

Tosi & al. (2004) reported that the morphological features of *Conidiobolus antarcticus* to some extent resembled those of *C. megalotocus* Drechsler, *C. thromboides* Drechsler, *C. bangalorensis* Sriniv. & Thirum., and *C. lamprauges* Drechsler, but our analysis of sequences from three gene regions reveals that *C. antarcticus* is clearly not closely related to *C. thromboides*, *C. bangalorensis*, or *C. lamprauges*. Instead, the phylogenetic tree showed a very close relationship between *C. antarcticus* and *C. osmodes*.

TABLE 2 provides a comparative summary of the morphology of *C. antarcticus* (from Tosi & al. 2004) and *C. osmodes* (from Drechsler 1954). These two species are morphologically similar in colony diameter, hyphal diameter, primary conidial size and shape, and zygospore formation, size, and shape of the zygospore; there are only slight differences in vegetative

Table 2. Morphological comparison of *Conidiobolus antarcticus* and *C. osmodes* (data from Tosi et al. 2004 and Drechsler 1954)

Character	*C. antarcticus*	*C. osmodes*
Colony diameter	c. 42 mm in 5 days	c. 30 mm in 4 days
Hyphal diameter	8–(10)–12 µm	4–12 µm
Hyphal segments	Continuous, but disjointed putting forth (3–4) short diverticulate branches	Becoming septate and noncontinuous
Primary conidia	Pyriform to globose, 25–31 × 20–25 µm, with 5 µm papilla	Globose to obovoid, 25–37 × 22–30 µm, with 2–5 µm papilla
Zygospores	Globose, 25–40 µm, smooth, thick-double walled (2.5–5 µm)	Globose to ellipsoidial, 13–37 µm, sometimes smooth but usually bearing smallish ridges, thick-double walled (2–6.5 µm)
Zygospore formation	Conjugation between two segments of separate hyphae or same hypha	Between conjugating cell of different or same hyphae or hyphal bodies
Odor	Not reported	Benzene hexachloride

appearance and colony odor. *Conidiobolus osmodes* RECF 4447 was checked for key morphological features, including colonial diameter (c. 33 mm in 4 d), primary conidia (25–38 × 22–33 µm), and zygospores (25–30 µm with thick-double wall 3–5 µm). These features resembled those of *C. antarcticus*.

We consider that the relevant morphological characteristics and sequences from three genes provide sufficient evidence to place *C. antarcticus* and *C. osmodes* in synonymy. Consequently, our taxonomic proposal is:

Conidiobolus osmodes Drechsler, Amer. J. Bot. 41: 571. 1954.
= *Conidiobolus antarcticus* S. Tosi, Caretta & Humber, Mycotaxon 90(2): 344. 2004.

Acknowledgments

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Cladosporium hebeiense sp. nov., pathogenic on grape leaves in China

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Abstract—A new fungus in the *Cladosporium herbarum* complex, *Cladosporium hebeiense*, was isolated from leaf spots of *Vitis vinifera* in China. It produced short 0–1-septate ramoconidia that distinguished it from similar species. A multigene phylogenetic analysis based on the internal transcribed spacer region (ITS), partial translation elongation factor 1-alpha (TEF 1-α) gene, and the actin gene (ACT) supported *C. hebeiense* as separate from the closely related *C. ramotenellum*. Pathogenicity testing confirmed that *C. hebeiense* is a pathogen contributing to grape leaf spot disease.

Key words—*Cladosporiaceae, Capnodiales,* phylogeny, taxonomy,

Introduction

Cladosporium Link is one of the largest and most heterogeneous hyphomycete genera encompassing more than 772 names (Dugan & al. 2004). These fungi are widely distributed throughout the world and have been isolated from plants, soil, food, textile, paint, and other organic materials (Bensch & al. 2012, 2015; Crous & al. 2014). Some *Cladosporium* species are capable of causing human and livestock diseases, and several, including *C. tenuissimum*

Cooke and *C. macrocarpum* Preuss, have been recorded as saprophytes from grapevines in China (Zhang 2003).

At present, several leaf spot diseases of grapevine have been reported in China, caused by *Sultanimyces vitiphyllus* (Speschnew) Videira & Crous (Fan & al. 2007, Song & al. 2014); *Pseudocercospora vitis* (Lév.) Speg. (Chai 2001) and *Passalora dissiliens* (Duby) U. Braun & Crous (Wang & al. 2002, Liang & al. 2010); *Acrospermum viticola* Ikata & Hitomi (Yao 2015); *Septoria ampelina* Berk. & M.A. Curtis, *Grovesinia moricola* (I. Hino) Redhead, and *Pseudopezicula tracheiphila* (Müll.-Thurg.) Korf & W.Y. Zhuang (Wang 2009); and *Neopestalotiopsis vitis* Jayaward. & al. (Jayawardena & al. 2016). Wilcox & al. (2015) have reported several additional grape leaf spot pathogens from other countries, e.g., *Briosia ampelophaga* Cavara, *Aureobasidium pullulans* (de Bary & Löwenthal) G. Arnaud, *Rhytisma vitis* Schwein., *Cladosporium viticola* Ces., *Asperisporium minutulum* (Sacc.) Deighton, and *Passalora heterosporella* U. Braun & Crous.

Here we describe a new *Cladosporium* pathogen, obtained from grape leaf spot in Hebei Province, China. Both morphological and molecular techniques were utilized to evaluate its taxonomic placement.

Materials & methods

Isolates & morphology

Grapevine leaf spot samples were collected from the greenhouse at Changli County, Hebei Province, China in 2016. All the symptomatic leaves were surface-sterilized with 70% ethanol for 1 min, then washed in sterilized water three times, and small pieces (<25 mm^2) of the diseased tissues were placed on potato dextrose agar (PDA) and incubated at 25°C in a 12–12 h light-dark artificial incubator until fungi were observed. Single spore isolations were used to obtain pure cultures as described in Chomnunti & al. (2014). The purified strains were re-cultured on PDA for 14 d at 25°C to observe colony morphologies (Sandoval-Denis & al. 2016). The conidia and conidiophores were photographed using an Axio Imager Z2 photographic microscope, and measurements were taken using ZEN PRO 2012 software. Thirty conidia were measured to calculate size range and average value. Living cultures of the two isolated strains were stored in the culture collection at Beijing Academy of Agricultural and Forestry Sciences, Beijing, China (JZB) and in Guizhou University Culture Collection, Guiyang, China (GUCC); the holotype specimen was conserved as a dried agar culture, in the Herbarium of Beijing Academy of Agricultural and Forestry Sciences (JZBL).

DNA extraction & PCR amplification

Genome DNA was extracted according to Damm & al. (2008). Polymerase chain reactions (PCR) were conducted using C1000 Touch™ thermal Cycler according to

White & al. (1990) with gDNA as the template. The three genes amplified were the internal transcribed spacer region (ITS; using primers ITS4/ITS5, White & al. 1990), partial translation elongation factor 1-alpha gene (TEF 1-α; using primers TEF1-728F/TEF-2, O'Donnell & al. 1998), and the actin gene (ACT; using primers ACT-512F/ACT-783R, Bensch & al. 2012). The PCR conditions were as follows—for ITS: initial denaturation for 3 min at 94°C, followed by 34 cycles of denaturation for 30 s at 94°C, 30 s of annealing at 58°C, 90 s elongation at 72°C, and a final extension for 10 min at 72°C; for TEF 1-α and ACT: initial denaturation for 3 min at 94°C, followed by 40 cycles of denaturation for 30 s at 94°C, 30 s of annealing, 90 s elongation at 72°C, and a final extension for 10 min at 72°C. The annealing temperatures used in the cycling program were 54°C for TEF 1-α, and 52°C for ACT. Following PCR amplification, products were visualised on 1% agarose gel stained with ethidium bromide under UV light using a Gel Doc™ XR+ Molecular Imager. The PCR products were sequenced at Beijing Sunbiotech Co. Ltd.

Phylogenetic analysis

The sequences results were analyzed by BLASTn of National Center for Biotechnology Information (NCBI), and then the relevant reference sequences were downloaded from GenBank (Table 1). The ITS, TEF 1-α, and ACT sequences were aligned using MAFFT v.7 (Katoh & Toh 2008, http://mafft.cbrc.jp/alignment/server/), and manually aligned using BioEdit when necessary (Hall 1999). Then aligned sequences were converted into .nexus format using ALTER alignment online web tool (http://sing.ei.uvigo.es/ALTER/). Maximum parsimony (MP) analysis was conducted using Phylogenetic Analysis Using Parsimony (PAUP) v.4.0b10 (Swofford 2002). The phylogenetic tree was inferred using heuristic search option with tree bisection reconnection (TBR) branch swapping and 1000 random sequence additions, branches of zero length were collapsed and all parsimonious trees were saved. Descriptive statistics included tree length (TL), consistency index (CI), retention index (RI), rescaled consistence index (RC) and homoplasy index (HI). Bootstrap analyses (Hillis & Bull 1993) were based on 1000 replications.

Bayesian analyses were performed in MrBayes v.3.0b4 (Ronquist & Huelsenbeck 2003). The following models were applied: JC+G for the ITS gene region, TrNef+G for the TEF 1-α gene region and TrN+I+G model was selected for ACT gene. The Markov Chain Monte Carlo sampling (MCMC) analysis was conducted with four simultaneous Markov chains. They were run for 1,000,000 generations; sampling the trees at every 100th generation. From the 10,000 trees obtained, the first 2000 representing the burn-in phase were discarded. The remaining 8000 trees were used to calculate posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01) (Crous & al. 2006). Sequences derived in this study have been deposited in GenBank (Table 1).

Pathogenicity test

Young and healthy detached *Vitis vinifer*a L. 'Summer Black' leaves collected from the greenhouse were used to test for pathogenicity. Leaves were disinfected with 75%

Table 1. Sequences of *Cladosporium* used in the molecular analyses.

Species	Isolate	GenBank accession numbers			Reference
		ITS	TEF1-α	ACT	
C. aggregatocicatricatum	CBS 140493	KT600448	KT600547	KT600645	Bensch & al. 2015
C. allicinum	CBS 121624	EF679350	EF679425	EF679502	Schubert & al. 2007
C. allii	CBS 101.81	JN906977	JN906983	JN906996	Bensch & al. 2012
C. angustiherbarum	CBS 140479	KT600378	KT600475	KT600574	Bensch & al. 2015
C. antarcticum	CBS 690.92	EF679334	EF679405	EF679484	Schubert &al. 2007
C. arthropodii	CBS 124043	JN906979	JN906985	JN906998	Bensch & al. 2012
C. basiinflatum	CBS 822.84	HM148000	HM148241	HM148487	Bensch &al. 2010
C. echinulatum	CBS 123191	JN906980	JN906987	JN906999	Bensch & al. 2012
C. floccosum	CBS 140463	LN834416	LN834512	LN834600	Sandoval-Denis & al. 2016
C. herbaroides	CBS 121626	EF679357	EF679432	EF679509	Schubert & al. 2009
C. herbarum	CBS 121621	EF679363	EF679440	EF679516	Schubert & al. 2009
C. iridis	CBS 138.40	EF679370	EF679447	EF679523	Schubert & al. 2009
C. limoniforme	CBS 113737	KT600396	KT600493	KT600591	Bensch & al. 2015
C. macrocarpum	CBS 121623	EF679375	EF679453	EF679529	Schubert & al. 2009
C. ossifragi	CBS 842.91	EF679381	EF679459	EF679535	Schubert & al. 2009
C. paralimoniforme	CGMCC3.18103	KX938393	KX938409.	KX938376	Ma & al. 2017

C. phlei	CBS 358.69	JN906981	JN906991	JN907000	Bensch & al. 2012
C. prolongatum	CGMCC3.18035	KX938395	KX938411	KX938378	Ma & al. 2017
C. pseudiridis	CBS 116463	EF679383	EF679461	EF679537	Schubert & al. 2009
C. ramotenellum	CBS 121628	EF679384	EF679462	EF679538	Schubert & al. 2007
C. rhoicola	CBS 140492	KT600440	KT600539	KT600637	Bensch & al. 2015
C. sinuosum	CBS 121629	EF679386	EF679464	EF679540	Schubert & al. 2007
C. soldanellae	CPC 13153	JN906982	JN906994	JN907001	Bensch & al. 2012
C. spinulosum	CBS 119907	EF679388	EF679466	EF679542	Schubert & al. 2007
C. subinflatum	CBS 121630	EF679389	EF679467	EF679543	Schubert & al. 2007
C. subtilissimum	CBS 113754	EF679397	EF679475	EF679551	Schubert & al. 2007
C. tenellum	CBS 121634	EF679401	EF679479	EF679555	Schubert & al. 2007
C. tuberosum	CBS 140693	LN834417	LN834513	LN834601	Sandoval-Denis & al. 2016
C. variabile	CBS 121635	EF679402	EF679480	EF679556	Schubert & al. 2007
C. verruculosum	CGMCC3.18099	KX938389	KX938405	KX938372	Ma & al. 2017
C. hebeiense	**JZB390001 [T]**	**MG516597**	**MG516595**	**MG516593**	**This paper**
	JZB390005	**MG516598**	**MG516596**	**MG516594**	**This paper**
C. colombiae	CBS 274.80B	FJ936159	FJ936163	FJ936166	Schubert & al. 2009
C. langeronii	CBS 189.54	DQ780379	JN906990	EF101357	Zalar & al. 2007

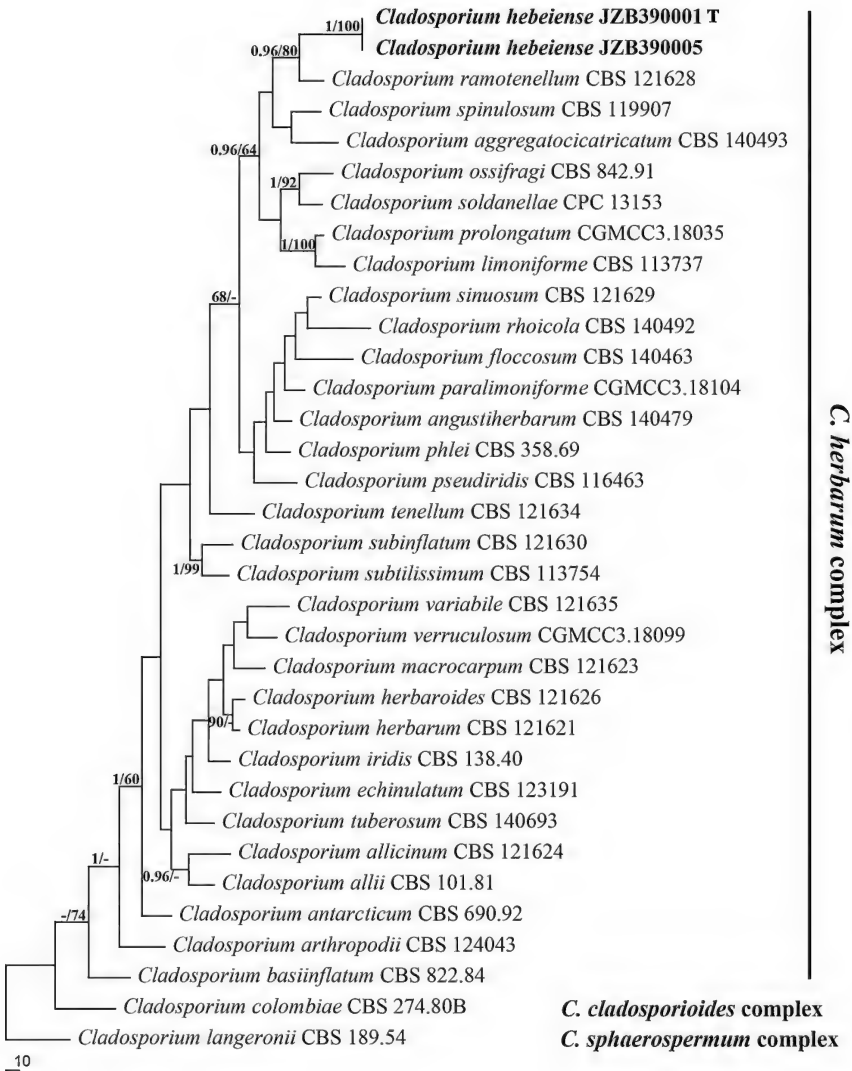

Fig. 1. Phylogram based on maximum parsimony and Bayesian analyses with combined ITS, TEF1-α, and ACT sequence data of *Cladosporium* species. Bootstrap values >50% and posterior probabilities >0.96 are indicated near the nodes. Sequences from our new species are set in bold font. The scale bar indicates 10 changes. The tree is rooted with *C. langeronii* [*C. sphaerospermum* complex] and *C. colombiae* [*C. cladosporioides* complex].

alcohol for 30 s, washed with sterile water 3 times, and then placed in sterile operating platform for drying. We re-cultured the isolates on PDA for 7 d, prepared a 1×10^5/ml spore suspension, and conducted the pathogenicity test using three leaves per isolate with the drip method. Leaves were inoculated using two inoculation methods: non-wounded and wounded, with 4 inoculation points on each leaf (2 inoculation points on each side) with a spore suspension of 20 µl. Inoculated leaves were placed into an inoculation box for moisturizing, and the box was placed in an incubator at 25°C for 12 h light/12 h dark. The pathogenic experiment was repeated 3 times, with sterile water serving as the negative control. After the onset of disease symptoms, the fungus was re-isolated, fulfilling Koch's postulates.

Phylogenetic results

The phylogenetic tree constructed by MP comprised 30 sequences from the *Cladosporium herbarum* complex (Bensch & al. 2012, 2015), with *Cladosporium langeronii* (CBS 189.54; *C. sphaerospermum* complex) and *C. colombiae* (CBS 274.80B; *C. cladosporioides* complex) as the outgroup. After the three gene regions (ITS, TEF 1-α, and ACT) were combined, the final alignment was 1048 characters long (ITS: 1–478, TEF 1-α: 479–859, ACT: 860–1048). The concatenated alignment contained 229 parsimony informative characters, 707 constant characters, and 112 variable and parsimony uninformative characters. One (TL = 1259, CI = 0.448, RI = 0.529, RC = 0.237, HI = 0.552) of 1000 equally parsimonious trees is shown in Fig. 1. Within the *Cladosporium herbarum* complex, our two isolates (JZB390001, JZB390005) clustered together with maximum parsimony bootstrap (MP) = 100% and Bayesian posterior probability (PP) = 1. Our isolates clustered with their sister taxon, *C. ramotenellum* K. Schub. & al. with MP = 80% and PP = 0.96.

Taxonomy

Cladosporium hebeiense C.X. Wang, Xing H. Li & Yan M. Wei, **sp. nov.** Fig. 2

Index Fungorum IF553970

Differs from *Cladosporium ramotenellum* by its shorter, wider ramoconidia with fewer septa.

Type: China, Hebei Province, Changli County, on leaf spot of greenhouse *Vitis vinifera* (*Vitaceae*), November 2016, Xinghong Li (Holotype, JZBL390001; ex-holotype cultures, JZB390001, GUCC3075; GenBank MG516597, MG516595, MG516593).

Etymology: Referring to the collection locality in Hebei Province.

Colonies: On PDA 45–55 mm diam after 14 d at 25°C, moderate green to dark green, flat to umbonate and slightly folded, velvety with white cottony centre and regular margin; reverse olive brown. On OA 43–47 mm diam after

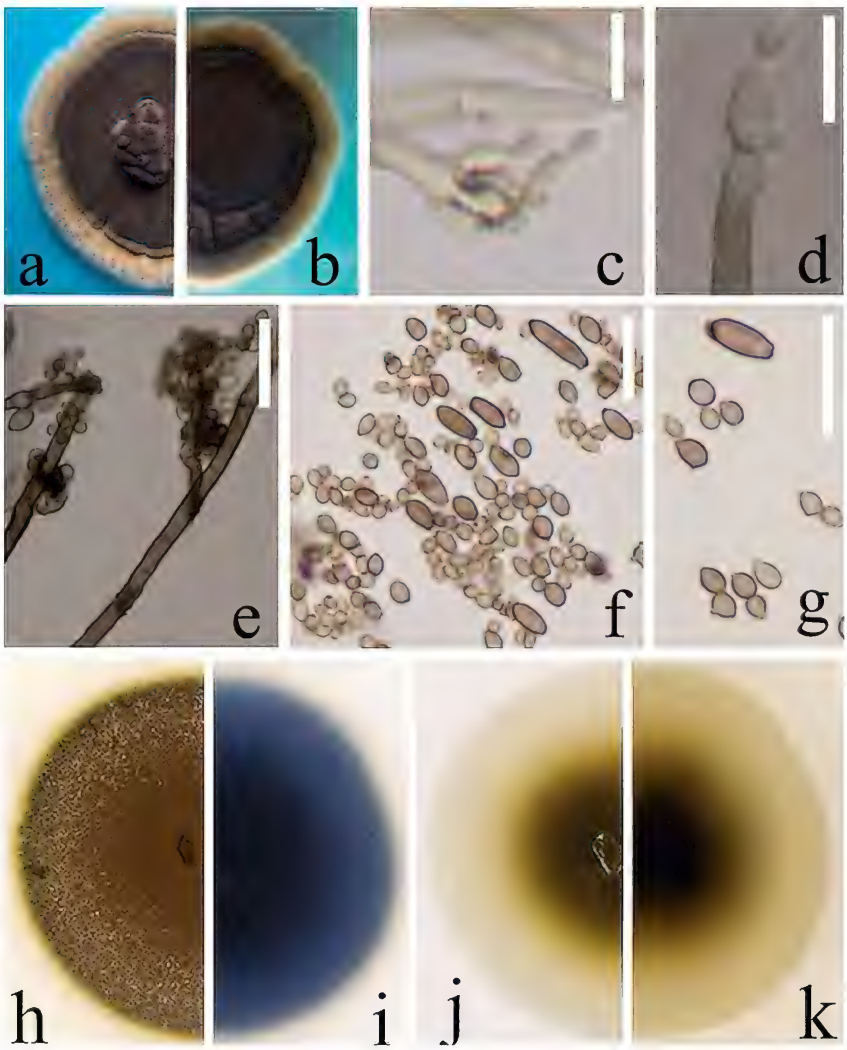

Fig. 2. *Cladosporium hebeiense* (JZB3900005, ex-holotype). a, b. Colonies on PDA (a-obverse, b-reverse) at 25°C; c, d. Sporulation with primary conidia on PDA; e. Conidiophores and conidia on PDA; f, g. Two kinds of conidia on PDA; h, i. Colonies on OA (a = obverse, b = reverse) at 25°C; j, k. Colonies on SNA (a = obverse, b = reverse) at 25°C. Scale bars: c, e, f = 5 μm; d, g = 10 μm.

14 d at 25°C, olive green or olive brown, nearly round, powdery, reverse dark olive green to black. On SNA 32–34 mm diam after 14 d at 25°C, yellowish-brown to dark brown, aerial hyphae not obvious, nearly round, reverse back

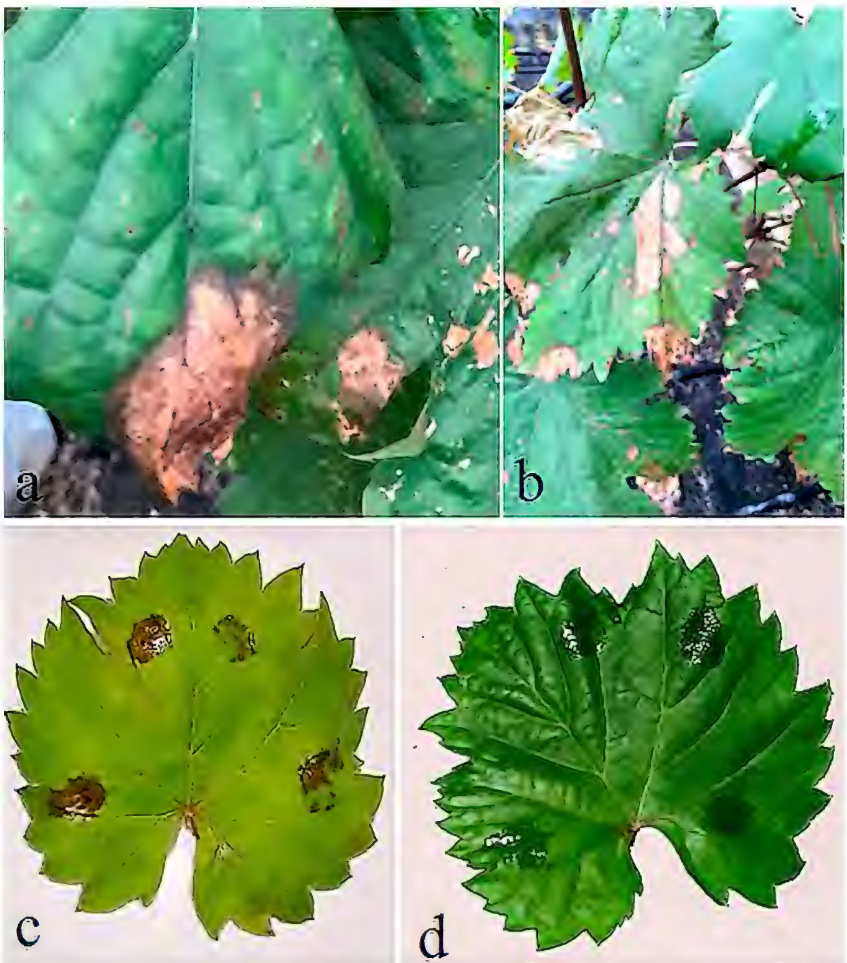

Fig. 3. Symptoms of grape leaf spot disease associated with *Cladosporium hebeiense*. a, b. Field symptoms of grape leaf spot; c. Symptoms on in vitro grape leaves, 7 d after inoculation with isolate JZB3900001. d. In vitro grape leaves, 7 d after control inoculation with sterile water.

brown to dark brown. Mycelium superficial and immersed, hyphae septate, branched, 1.5–3 µm wide, sub-hyaline to pale brown, smooth. Conidiophores erect, flexuous, sub-cylindrical, septate, mostly unbranched, ≤150 × 3–3.5 µm, pale to medium olivaceous brown, smooth. Conidiogenous cells terminal, cylindrical, nodulose, 18–20 × 3–4.5 µm, smooth and thick-walled, bearing up to 2–3 conspicuous, refractive, slightly darkened conidiogenous loci of 2–2.5

µm diam. Ramoconidia 0–1-septate, subcylindrical to cylindrical, 7.5–20 × 4–5.5 µm (mean = 10.24 × 4.54 µm, n = 30), smooth or finely verruculose. Conidia forming branched chains with ≤5–7 conidia at the unbranched terminus, pale green-brown, smooth- and thick-walled, with protuberant and darkened conidial hila; small terminal conidia aseptate, obovoidal to short ellipsoidal, 3–8 × 3–5 µm (mean = 5.48 × 3.88 µm, n = 30).

Additional material examined: **CHINA, Hebei Province, Changli County**, on leaf spot of greenhouse *Vitis vinifera*, November 2016, Xinghong Li (cultures (JZB390005, GUCC3076; GenBank MG516598, MG516596, MG516594).

Pathogenicity

This grape leaf spot is primarily characterized as light brown spots with a darker edge, circular, oval, or irregular in shape, mainly concentrated in the relatively old leaves (Fig. 3). The leaves begin to show symptoms at 3 d. Symptoms were similar to those of field acquired leaf spot disease at 7 d. No symptoms were observed on the control (Fig. 3). After Koch's postulates, re-isolated cultures, and morphological observation of ramoconidia and conidia confirmed the pathogen was *Cladosporium hebeiense*.

Discussion

As can be seen from the phylogenetic tree (Fig. 1), *Cladosporium hebeiense* falls within the *C. herbarum* complex and is closely related to *C. ramotenellum* with strong support (MP = 80%, PP = 0.96). However, *C. ramotenellum* differs from *C. hebeiense* by its longer, narrower ramoconidia with more numerous septa (<47 × 2–4 µm, 0–1(–4)-septate; Bensch & al. 2012, Schubert & al. 2007). Based on morphological comparison and phylogenetic analysis, we confirm that *C. hebeiense* is a new taxon in the *C. herbarum* complex.

Cladosporium species are very common fungi that cause leaf spot diseases in many plants such as rock lily, wheat, *Vigna umbellata, Citrus sinensis,* and *Colocasia esculenta* (Bensch & al. 2012, Sandoval-Denis & al. 2016). However, at present there are no other reports from China of grape leaf spots caused by *Cladosporium*. Our report not only adds a new *Cladosporium* species, but also promotes the research progress of grape leaf spot in China. Our next step will be to investigate the main grape producing areas in China to evaluate the impact of *C. hebeiense* on Chinese grapes.

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Eighteen species of *Graphidaceae* new to Nepal

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Abstract—Eighteen graphidoid lichen species in *Diorygma, Graphis, Pallidogramme,* and *Phaeographis* are reported as new records from Nepal. Diagnostic characters of each species are discussed and supported by chemistry, ecology, and distribution. An identification key is provided to the 24 graphidaceous species recorded from Nepal.

Key words—Kathmandu, lichenized fungi, lirellate, taxonomy

Introduction

Molecular studies have provided a broad framework for *Graphidaceae* and produced numerous nomenclatural changes and rearrangements. These have resulted in the fusion of several concepts, with some old or obsolete taxa resurrected and many new taxa described (Staiger & Kalb 1999; Staiger 2002; Staiger & al. 2006; Lücking & al. 2009, 2013; Mangold & al. 2008, 2009; Rivas Plata & Lumbsch 2011, Rivas Plata & al. 2012a,b, 2013; Kraichak & al. 2014; Lumbsch & al. 2014a,b). As currently accepted, *Graphidaceae* represents the second largest lichen family, with 2161 species distributed among 79 genera (Lücking & al. 2017).

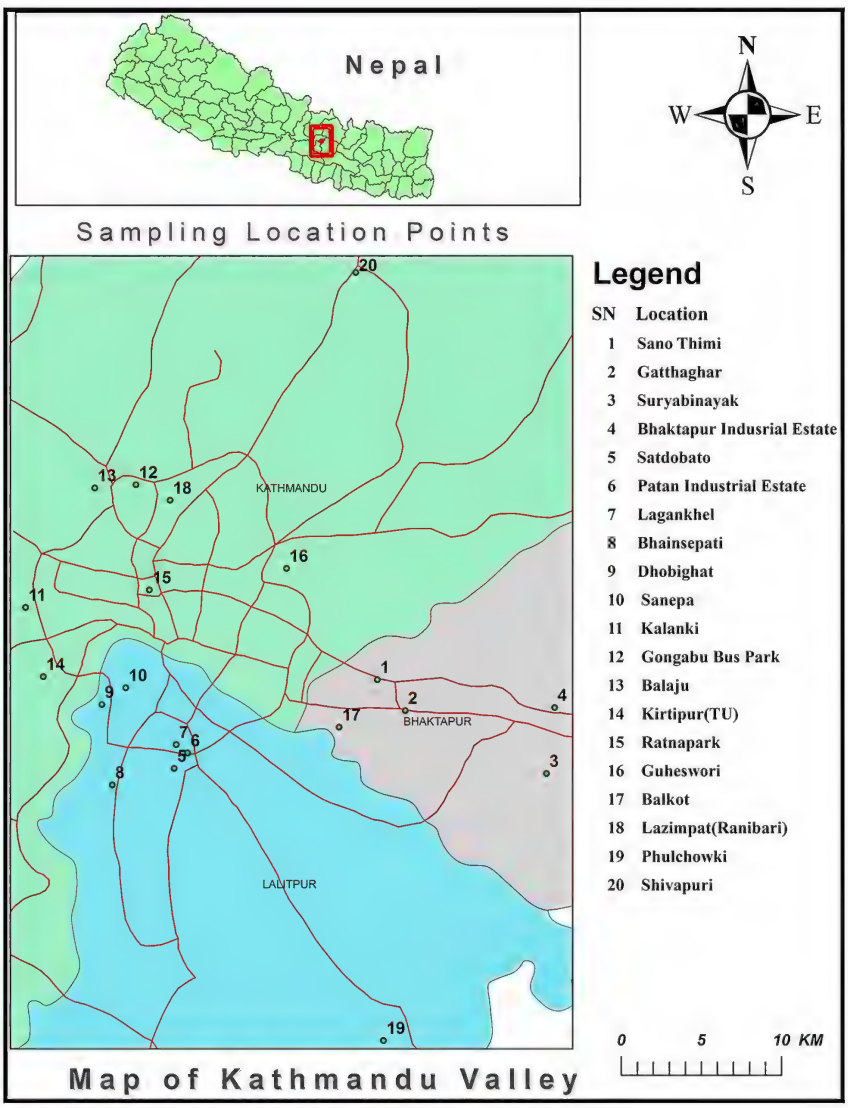
Lichenological studies in Nepal have been conducted by primarily western and a few native lichenologists (Lamb 1966; Bystrek 1969; Abbayes 1974; Poelt 1974; Jahns & Seelen 1974; Kurokawa 1974; Mitchell 1974; Schmidt 1974; Vězda & Poelt 1975; Awasthi 1986, 1991, 2007; Awasthi & Mathur 1987; Sharma 1995; Baniya & al. 2001; Olley & Sharma 2013), and a total of 820 species of lichenized-fungi have been recorded from the country (Olley & Sharma 2013, Rai et al 2016). However, the crustose lichens have been little studied, particularly the family *Graphidaceae* with lirellate taxa, for which only six species—*Glyphis cicatricosa* Ach., *Graphis chlorotica* A. Massal., *G. proserpens* Vain., *G. scripta* (L.) Ach., *G. tenella* Ach., and *Platythecium pyrrhochroum* (Mont. & Bosch.) Z.F. Jia & Lücking—have been recorded from Nepal (Olley 2008, Olley & Sharma 2013, Rai & al. 2016). The present study covers species of graphidoid (lirellate) lichens collected from central Nepal and reported as new to the country.

Including the 18 new records described in this paper, 24 species in *Graphidaceae* are now known from Nepal. We refrain from including *Phaeographina nepalensis* D.D. Awasthi & Kr.P. Singh described from Nepal (Awasthi & Singh 1973), because its identity is uncertain; it may represent a *Graphis* species with striate labia, although brown ascospores are rare in the genus. Two other names previously reported from Nepal are included as synonyms: *Graphis subglauconigra* Nagarkar & Patw. has been synonymised with *G. tenella* (Lücking & al. 2009); and *Phaeographina pyrrhochroa* is a homotypic synonym of the new combination *Platythecium pyrrhochroum* (Jia & Lücking 2017). Three newly reported species extend across Asia from the Neotropics to the Eastern Palaeotropics. The amount of interesting material in our collections suggests an extraordinary diversity of *Graphidaceae* in Nepal and indicates the need for further lichenological investigations in unexplored areas of the country.

Materials & methods

The study sites were located in different areas around Kathmandu Valley in the Bagmati Zone of Central Nepal (Map 1). The valley is roughly 650 km^2 with elongated east west; its average elevation is c. 1350 m and annual precipitation averages 1343 mm (Shakya & al. 2014).

Initially, a GIS based sampling map of Kathmandu was designated (Map 1) to locate the 20 study sites. Samples were collected from all the sites based on availability of lichens on barks. The collected material was processed for curation using standard lichenological procedures (Awasthi 2000), and the voucher specimens



Map 1. Sampling areas in the Kathmandu Valley of Nepal.

were deposited at the National Herbarium Laboratory, Godavari, Kathmandu, Nepal (KATH). Specimens were examined and identified at the Lichenology laboratory, CSIR-National Botanical Research Institute, Lucknow, India. Diagnostic characters

were observed with standard light microscopy techniques using a LEICA™ S8APO stereomicroscope and LEICA™ DM500 optical microscope. Thallus and ascocarp sections were mounted in water, 10% KOH, and lactophenol blue; Lugol's solution was used to check amyloidity (I+ or I–). All measurements were made in water. Chemical constituents were identified by spot color reaction tests and thin layer chromatography (TLC; solvent system A) following published protocols (Elix & Ernst-Russel 1993, Orange & al. 2001). Measurements of hymenium, exciple, asci, and ascospores (5-10 ascospores measured per sample) were made on 5–10 thin, hand-cut apothecial sections per collection. Specimen identifications, nomenclatural changes, and new records were validated against relevant keys, monographs, and checklists (Archer 2005; Lücking & al. 2009; Singh & Sinha 2010; Joshi et al. 2013a,b; Olley & al. 2013; Mishra & al. 2016). Terms used to define lirellae morphs follow Lücking & al. (2009).

Taxonomy

Diorygma hieroglyphicum (Pers.) Staiger & Kalb,
Symb. Bot. Upsal. 34(1): 151, 2004. PL. 1A

Thallus corticolous, crustose, greenish-white, smooth to uneven, 120–140 µm thick; photobiont *Trentepohlia*; ascomata lirellate, lirellae emergent to semi-emergent, white, straight to curved, simple to branched, 2.0–3.5 × 0.3–0.5 mm, with indistinct thalline margin; disc broad, concealed, flat to concave, white pruinose, 0.2–0.4 mm in diam.; proper exciple hyaline to pale-brown, divergent, poorly developed; hymenium hyaline, clear; asci 1-spored; ascospores hyaline, amyloid (I+ blue), muriform, with unequal locules, 95–150 × 30–40 µm.

CHEMISTRY—Thallus K+ yellow orange, Pd+ reddish, C–, KC–. Stictic acid detected with TLC.

ECOLOGY—*Diorygma hieroglyphicum* was found growing on the barks of *Alnus nepalensis* D. Don (*Betulaceae*) and *Castanopsis indica* (Roxb. ex Lindl.) A. DC. (*Fagaceae*) at 1600–1900 m near the buffer zone of Shivapuri National Park and of Phulchowki forest area.

SPECIMENS EXAMINED – **NEPAL. BAGMATI ZONE:** Shivapuri National Park, 27°35′05″N 85°22′42″E, alt. 1626 m, on tree bark, 1 December 2016, Neena Karmacharya 16–134 (KATH); Phulchowki, 27°34′47″N 85°23′09″E, alt. 1936 m, on tree bark, 1 December 2016, Neena Karmacharya 16–148 (KATH).

COMMENTS—Our Nepalese specimens corresponded closely to previously published descriptions of *D. hieroglyphicum*. This pantropical species has been recorded in Nepal, India, Vietnam, Australia, Cameroon, New Caledonia, Tanzania, Indonesia, Singapore, Papua New Guinea, the Philippines, Vanuatu, and the Solomon Islands (Archer 2009, Kalb & al. 2004, Joshi & al. 2013a).

Diorygma junghuhnii (Mont. & Bosch.) Kalb, Staiger & Elix, Symb. Bot. Upsal. 34(1): 157, 2004. Pl. 1B

Thallus corticolous, crustose, white, creamy, pale-gray or greenish-gray, rough, dull, 75–90 µm thick; photobiont *Trentepohlia;* ascomata lirellate, lirellae emergent, curved, simple to branched 2–3.5 × 0.5–1 mm with crenate thalline margin; disc concealed, brown to dark brown, white pruinose; proper exciple hyaline to pale-brown, slightly divergent, poorly developed; hymenium hyaline, clear; asci 1–2-spored; ascospores hyaline, amyloid (I+ violet-blue), muriform (25–31 × 6–10 locular) measuring 84–134 × 29–42 µm.

Chemistry—Thallus K+ yellow orange, Pd+ yellow-orange, C–, KC–. Norstictic and constictic acids detected with TLC.

Ecology—*Diorygma junghuhnii* was collected with many other members of *Parmeliaceae* and *Physciaceae* at c. 1600 m in the Phulchowki forest area.

Specimen examined – **NEPAL. Bagmati zone:** Phulchowki, 27°35′05″N 85°22′42″E, alt. 1626 m, on tree bark, 19 December 2016, Neena Karmacharya 16–089A (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *D. junghuhnii,* the most common *Diorygma* species. This pantropical species has previously been reported from India, China, Vietnam, Japan, the Philippines, Indonesia, Australia, New Caledonia, Tanzania, Brazil, Costa Rica, Guatemala, Paraguay, Solomon Islands, Tahiti, and Thailand (Kalb & al. 2004, Joshi & al. 2013b).

Graphis antillarum Vain., Hedwigia 38: 255, 1899. Pl. 1C

Thallus corticolous, crustose, whitish-gray, rough; photobiont *Trentepohlia;* ascomata lirellate, lirellae appear whitish-gray due to broken thalline cover studded in furrows of labia, erumpent, elongate, curved, simple to irregularly branched (*tenella*-morph), 2–8 × 0.2 mm in size with thick lateral thalline margin; labia striate; proper exciple laterally carbonized; hymenium hyaline, clear; asci 2–8-spored; ascospores hyaline, amyloid (I+ blue), muriform, 30–60 × 17–25 µm.

Chemistry—Thallus K+ yellow turning red, Pd+ yellow-orange, C–, KC–; norstictic acid detected with TLC.

Ecology—*Graphis antillarum* was collected from the bark of *Rhododendron* spp. (*Ericaceae*) in the Shivapuri National Park at c. 1800 m.

Specimen Examined – **NEPAL. Bagmati zone:** Shivapuri National Park, 27°46′49″N 85°22′56″E, alt. 1875 m, on tree bark, 11 February 2017, Neena Karmacharya 17–207C (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. antillarum*. This cosmopolitan species was previously reported from Germany and Costa Rica (Lücking & al. 2008, 2009).

Graphis breussii G. Neuwirth & Lücking, Lichenologist 41: 272, 2009. Pl. 1D

Thallus corticolous, crustose, rough, gray to whitish-gray with dull surface; photobiont *Trentepohlia;* ascomata lirellate, lirellae elongate, curved, prominent, simple to irregularly branched (*acharii*-morph), 6–10 × 0.3–0.5 mm, with apically thin complete and conspicuous thalline margin; labia striate, convergent; proper exciple completely carbonized, deeply incised with thalline cover; hymenium hyaline, clear; asci 4-spored; ascospores hyaline, muriform (7–11 × 1–2 locular), amyloid (I+ blue-violet), 30–45 × 9–12 µm.

Chemistry—Thallus K+ yellow turning red, Pd+ yellow-orange, C–, KC–. Norstictic acid detected with TLC.

Ecology—*Graphis breussii* was found growing among other foliose and crustose lichens on bark of *Alnus nepalensis* and collected from Phulchowki at c. 1600 m.

Specimen examined – **NEPAL. Bagmati zone:** Phulchowki, 27°35′05″N 85°22′42″E, alt. 1626 m, on tree bark, 19 December 2016, Neena Karmacharya 16–093C (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. breussii*. This new continental record was previously known from southern Venezuela (Lücking & al. 2009).

Graphis cincta (Pers.) Aptroot, Fl. Australia 57: 651, 2009. Pl. 1E

Thallus corticolous, crustose, off-white to pale-fawn, smooth, dull; photobiont *Trentepohlia;* ascomata lirellate, lirellae erumpent, numerous, scattered, elongate, straight to curved or sinuous, simple, 0.5–2.0 × 0.1–0.2 mm, with a conspicuous basal to lateral thalline margin (*lineola*-morph); disc concealed, rarely slightly open; labia entire; proper exciple laterally carbonized; hymenium hyaline, inspersed with oil droplets; asci 8-spored; ascospores hyaline, amyloid (I+ blue), transversely 7–11-septate, measuring 25–40 × 7–10 µm.

Chemistry—Thallus K+ yellow turning red, Pd+ yellow-orange, C–, KC–. Norstictic acid detected with TLC.

Ecology—*Graphis cincta* was collected from the bark of *Cinnamomum camphora* (L.) J. Presl (*Lauraceae*) near industrial site of Balaju in Kathmandu Valley at c. 1300 m.

Specimen examined – **NEPAL. Bagmati zone:** Balaju, 27°44′01″N 85°18′02″E, alt. 1310 m, on tree bark, 11 November 2016, Neena Karmacharya 16–046A (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. cincta.* It has a pantropical distribution and was previously reported from India, Australia, Paraguay, the Philippines, Taiwan, West Indies, Costa Rica, Japan, and Vanuatu (Singh & Sinha 2010; Archer 2009; Lücking & al. 2008, 2009).

Graphis cleistoblephara Nyl., Ann. Sci. Nat., Bot., Sér. 4, 20: 265, 1863. Pl. 1F

Thallus corticolous, crustose, gray to pale-gray, fawn colored, thin, smooth, dull; photobiont *Trentepohlia;* ascomata lirellate, lirellae erumpent, conspicuous, numerous, scattered, very short, unbranched, sessile, straight, 0.5–0.2 × 0.3–0.5 mm, with a thick conspicuous lateral thalline margin (*dussii*-morph); disc concealed; labia entire; proper exciple completely carbonized; hymenium: hyaline, clear; asci 2–4-spored; ascospores hyaline, amyloid (I+ blue), muriform (19–23 × 2–5 locular) measuring 85–120 × 15–20 µm.

Chemistry—Thallus K+ yellow turning red, Pd+ yellow-orange, C–, KC–. Norstictic acid detected with TLC.

Ecology—*Graphis cleistoblephara* occurs most commonly on the bark of *Acer oblongum* Wall. ex DC. (*Aceraceae*) at c. 1300 m in the Ranibari area of Kathmandu.

Specimen examined – **NEPAL. Bagmati zone:** Ranibari, 27°43′49″N 85°19′15″E, alt. 1326 m, on tree bark, 13 October 2016, Neena Karmacharya 16–016 (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. cleistoblephara.* The pantropical species has been recorded from India, Australia, China, Indonesia, Japan, Mexico, the Philippines, and Sri Lanka (Lücking & al. 2009, Singh & Sinha 2010).

Graphis galactoderma (Zahlbr.) Lücking, Lichenologist 41: 436, 2009. Pl. 1G

Thallus corticolous, crustose, white to pale-yellow, smooth to cracked; photobiont *Trentepohlia;* ascomata lirellate, lirellae erumpent to prominent, crowded, curved, simple to sparsely branched, 3–6 × 0.2 mm, with lateral thalline margin (*lineola*-morph); labia 1–4 striate; proper exciple apically (to peripherally) carbonized; hymenium hyaline, clear; asci 8-spored; ascospores hyaline, amyloid (I+ blue), muriform, 20–45 × 9–16 µm.

Chemistry—Thallus K+ yellowish, Pd+ orange, C–, KC–. Stictic acid detected with TLC.

Ecology—*Graphis galactoderma* was common among the *Quercus semecarpifolia* Sm. (*Fagaceae*) forest area in Phulchowki forest area above 2000 m.

Specimen examined – **NEPAL. Bagmati zone:** Phulchowki, 27°34′46″N 85°23′31″E, alt. 2156 m, on tree bark, 04 February 2017, Neena Karmacharya 17–158B (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. galactoderma.* The species is common in eastern paleotropics and has been recorded from the tropical regions of India and China (Lücking & al. 2009, Archer 2009).

Graphis leprographa Nyl., Acta Soc. Sci. Fenn. 26(10): 21, 1900. Pl. 1h

Thallus corticolous, crustose, white to yellowish-gray, smooth to uneven; photobiont *Trentepohlia;* ascomata lirellate, lirellae prominent, simple, crowded, very short, unbranched with thick lateral thalline margin (*dussii*-morph); labia entire; proper exciple completely carbonized; hymenium hyaline, clear; asci 1–2-spored; ascospores hyaline, amyloid (I+ blue), muriform, 60–110 × 15–30 µm.

Chemistry—Thallus K+ yellow turning red, Pd+ yellow-orange, C–, KC–. Norstictic acid detected with TLC.

Ecology—*Graphis leptographa* was found growing in association with some leprose taxa and collected from the bark of *Alnus nepalensis* at c. 1600 m in the buffer zone of the Shivapuri National Park.

Specimen examined – **NEPAL. Bagmati zone:** Shivapuri National Park, 27°47′30″N 85°22′15″E, alt. 1647 m, on tree bark, 19 December 2016, Neena Karmacharya 16–133A/c (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. leptographa.* It has pantropical distribution and recorded previously from Sri Lanka, Costa Rica, and Seychelles (Lücking & al. 2008, 2009).

Graphis lineola Ach., Lich. Univ.: 264, 1810. Pl. 1i

Thallus corticolous, crustose, whitish-gray, smooth, dull; photobiont *Trentepohlia*; ascomata lirellate, lirellae erumpent, numerous, simple to sparsely branched with lateral thalline margin (*lineola*-morph), 0.5–3 × 0.1–0.3 mm; disc concealed; labia entire; proper exciple laterally carbonized; hymenium hyaline, inspersed with oil-droplets; asci 8-spored; ascospores hyaline, amyloid (I+ blue), transversely 5–11-septate, 60–110 × 15–30 µm.

Chemistry—Thallus K–, Pd–, C–, KC–. No substances detected with TLC.

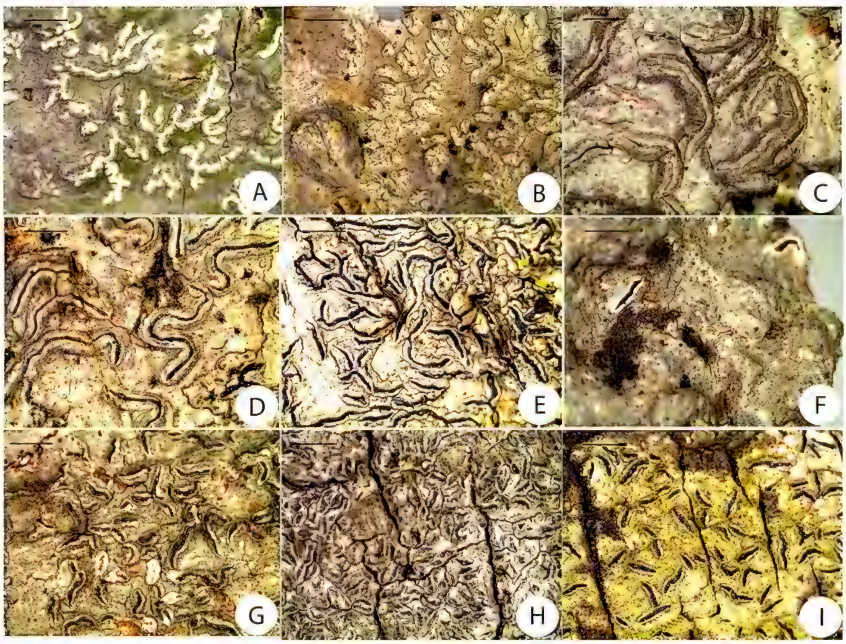


Plate 1. New records in *Graphidaceae* from Nepal. A. *Diorygma hieroglyphicum*; B. *D. junghuhnii*; C. *Graphis antillarum*; D. *G. breussii*; E. *G. cincta*; F. *G. cleistoblephara*; G. *G. galactoderma*; H. *G. leprographa*; I. *G. lineola*. Scale bars: A, B, G = 2 mm; C–F, H, I = 1 mm.

Ecology—*Graphis lineola* was collected from the bark of *Pinus roxburghii* Sarg. (*Pinaceae*) growing along the roadside at c. 1300 m in the Bhaisepati area of Kathmandu.

Specimen examined – **NEPAL. Bagmati zone:** Bhaisepati, 27°39′13″N 85°18′19″E, alt. 1344 m, on tree bark, 24 November 2016, Neena Karmacharya 16–109B (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. lineola,* which occurs commonly in the tropics and has been recorded from India, China, Costa Rica, Taiwan, West Indies, and Portugal (Lücking & al. 2009, Singh & Sinha 2010, Lepista & Aptroot 2016).

Graphis paradisserpens Sipman & Lücking, Fieldiana, Bot. 46: 96, 2008. Pl. 2a

Thallus corticolous, crustose, creamy white to pale-gray, smooth to uneven, dull; photobiont *Trentepohlia;* ascomata lirellate, lirellae erumpent, very long, curved, radiately branched, 2–8 × 0.2–0.3 mm, lacking or with basal thalline margin (*sorediosa*-morph); disc concealed; labia striate; proper exciple apically

(to peripherally) carbonized; hymenium hyaline, clear; asci 2–8-spored; ascospores hyaline, amyloid (I+ blue), submuriform (5–7 × 0–2 locular), 25–40 × 8–15 µm.

Chemistry—Thallus K–, Pd–, C–, KC–. No substances detected with TLC.

Ecology—*Graphis paradisserpens* was collected from the bark of *Alnus nepalensis* at c. 1600 m in the Phulchowki forest area.

Specimen examined – **NEPAL. Bagmati zone:** Phulchowki, 27°35′05″N 85°22′42″E, alt. 1626 m, on tree bark, 16 December 2016, Neena Karmacharya 16–093A/a (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. paradisserpens*. The species was previously reported from the neotropical regions of Costa Rica and the West Indies (Lücking & al. 2009). Our report represents a new record for Asia.

Graphis paraserpens Lizano & Lücking, Fieldiana, Bot. 46: 96, 2008. Pl. 2B

Thallus corticolous, crustose, gray to pale-gray, smooth to uneven; photobiont *Trentepohlia;* ascomata lirellate, lirellae emerged to erumpent, elongate, straight to flexuose, simple to irregularly branched with apically thin complete thalline margin (*symplecta*-morph), 2–5 × 0.2–0.3 mm; disc concealed; labia striate; proper exciple apically (to peripherally) carbonized; hymenium hyaline, clear; asci 2–6-spored; ascospores hyaline, amyloid (I+ blue), muriform, 30–40 × 9–12 µm.

Chemistry—Thallus K–, Pd–, C–, KC–. No substances detected with TLC.

Ecology—*Graphis paraserpens* was collected from the bark of *Schima wallichii* Choisy (*Theaceae*) at c. 1700 m in the forested area of Shivapuri National Park.

Specimen examined – **NEPAL. Bagmati zone:** Shivapuri National Park, 27°47′27″N 85°22′23″E, alt. 1706 m, on tree bark, 11 February 2017, Neena Karmacharya 16–182 (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. paraserpens*. The species is new to palaeotropical region and has been reported from Brazil and Costa Rica (Lücking & al. 2008, 2009).

Graphis pertricosa (Kremp.) A.W. Archer, Telopea 11: 73, 2005. Pl. 2C

Thallus corticolous, crustose, off-white to fawn, smooth to verrucose, dull; photobiont *Trentepohlia;* ascomata lirellate, lirellae erumpent, straight

to curved or sinuous, numerous, scattered, short and simple to sparsely branched with apically thin complete thalline margin (*negrosina*-morph), 1–2.5 × 0.1–0.3 mm; disc concealed; labia entire; proper exciple laterally carbonized; hymenium hyaline, inspersed with oil-droplets; asci 8-spored; ascospores hyaline, amyloid (I+ blue), muriform (6–9× 1–3 locular), 25–40 × 8–12 µm.

Chemistry—Thallus K+ yellow turning red, Pd+ reddish, C–, KC–. Norstictic acid detected with TLC.

Ecology—*Graphis pertricosa* was common on the bark of *Castanopsis indica* at c. 2000 m in the valley of the Phulchowki forest area.

Specimen examined – **NEPAL. Bagmati zone:** Phulchowki, 27°34′46″N 85°23′31″E, alt. 2156 m, on tree bark, 24 February 2017, Neena Karmacharya 16–163A (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. pertricosa.* The species is common in eastern palaeotropical countries including Australia (Lücking & al. 2009) and has also been reported from India, Indonesia, Malaysia, and Tanzania (Archer 2009, Singh & Sinha 2010).

Graphis pinicola Zahlbr., Symb. Sin. 3: 43, 1930. Pl. 2d

Thallus corticolous, crustose, white to whitish-gray, smooth to verrucose; photobiont *Trentepohlia;* ascomata lirellate, lirellae erumpent to prominent, short to elongate, straight to curved, sparsely and irregularly or branched with lateral thalline margin (*lineola*- or *deserpens*-morph), 1–4 × 0.1–0.3 mm; disc concealed; labia entire; proper exciple laterally carbonized; hymenium hyaline, clear; asci 8-spored; ascospores hyaline, amyloid (I+ blue), transversely 8–10-septate, 25–35 × 10–12 µm.

Chemistry—Thallus K–, Pd–, C–, KC–. No substances detected with TLC.

Ecology—*Graphis pinicola* was collected from the bark of *Schima wallichii*, where it was growing in association with *Graphis paraserpens* and other corticolous lichenized and non-lichenized fungi at c. 1700 m in the Shivapuri National Park of Kathmandu.

Specimen examined – **NEPAL. Bagmati zone:** Shivapuri National Park, 27°47′27″N 85°22′23″E, alt. 1706 m, on tree bark, 11 February 2017, Neena Karmacharya 17–200A (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. pinicola.* The pantropical species has been reported also from India, China, Bolivia, and Thailand (Lücking & al. 2009, Singh & Sinha 2010).

Graphis stenotera Vain., Ann. Acad. Sci. Fenn., Ser. A, 15: 243, 1921. Pl. 2E

Thallus corticolous, crustose, off-white to whitish-gray, smooth, dull, slightly rimose; photobiont *Trentepohlia;* ascomata lirellate, lirellae erumpent to prominent, scattered, straight, curved to sinuous, simple to sparsely branched lacking lateral thalline margin, 1–3 × 0.15–0.3 mm; disc concealed; labia striate (*striatula*-morph), non-pruinose; proper exciple apically carbonized; hymenium hyaline, clear; asci 8-spored; ascospores hyaline, amyloid (I+ blue), transversely 6–9-septate, 20–35 × 6–9 µm.

Chemistry—Thallus K+ yellow, Pd+ yellowish, C–, KC–. Stictic acid detected with TLC.

Ecology— *Graphis stenotera* was collected from the bark of *Alnus nepalensis* at c. 1600 m in the Phulchowki forest area.

Specimen examined – **NEPAL. Bagmati zone:** Phulchowki, 27°35′05″N 85°22′42″E, alt. 1626 m, on tree bark, 19 December 2016, Neena Karmacharya 16–093B (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. stenotera.* The tropical species has been reported from Nepal, India, Costa Rica, the Philippines, and Taiwan (Singh & Sinha 2010).

Graphis subvelata Stirt., Queensland Agric. J. 5: 488, 1899. Pl. 2F

Thallus corticolous, crustose, off-white to whitish-gray, smooth, dull, slightly rimose; photobiont *Trentepohlia;* ascomata lirellate, lirellae erumpent, curved or sinuous, short to elongate, simple to sparsely branched with lateral thalline margin (*lineola*- or *deserpens*-morph), 1–4 × 0.1–0.3 mm; disc concealed; labia entire; proper exciple laterally carbonized; hymenium hyaline, inspersed with oil-droplets; asci 8-spored; ascospores hyaline, amyloid (I+ blue), muriform (5–10 × 1–3 locular), 35–45 × 8–15 µm.

Chemistry—Thallus K–, Pd–, C–, KC–. No substances detected with TLC.

Ecology—*Graphis subvelata* was collected from the bark of *Schima wallichii* in association with other graphidoid lichens at c. 1800 m in Shivapuri National Park in Kathmandu.

Specimen examined – **NEPAL. Bagmati zone:** Shivapuri National Park, 27°47′21″N 85°22′36″E, alt. 1802 m, on tree bark, 11 February 2017, Neena Karmacharya 17–196B (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *G. subvelata.* The species has an eastern palaeotropical distribution in Nepal, New Zealand, the Philippines, Papua New Guinea, Thailand, and Australia (Archer 2009, Lücking & al. 2009).

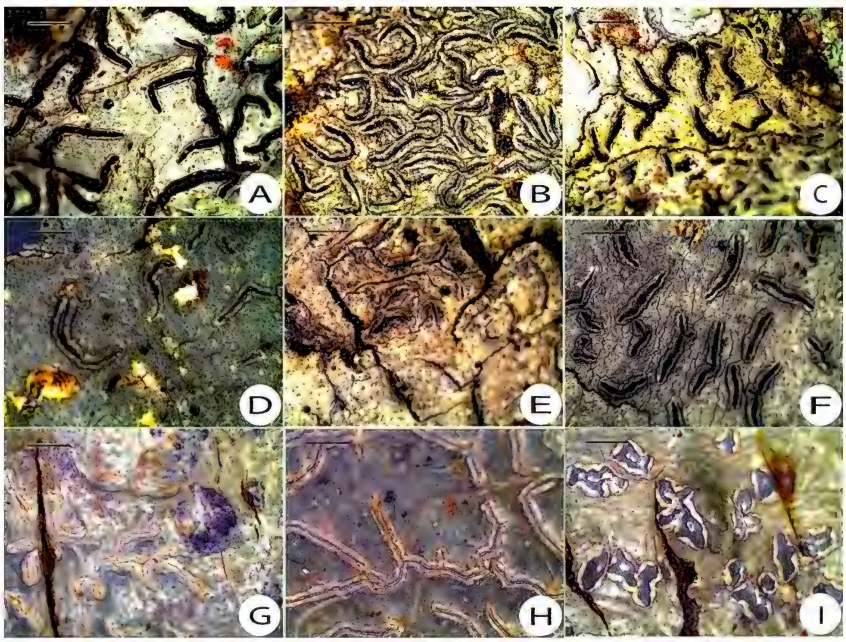


Plate 2. New records in *Graphidaceae* from Nepal. A. *Graphis paradisserpens*; B. *G. paraserpens*; C. *G. pertricosa*; D. *G. pinicola*; E. *G. stenotera*; F. *G. subvelata*; G. *Pallidogramme chrysenteron*; H. *P. divaricoides*; I. *Phaeographis leiogrammodes*. Scale bars = 1 mm.

Pallidogramme chrysenteron (Mont.) Staiger, Kalb & Lücking, Fieldiana, Bot. 46: 9, 2008. Pl. 2G

Thallus corticolous, crustose, off-white to pale-fawn, smooth, dull; photobiont *Trentepohlia;* ascomata lirellate, lirellae sessile, numerous, curved to sinuous, often branched, 1–5 × 0.4–0.6 mm; disc concealed; labia striate; proper exciple fan-shaped, yellowish-brown, convergent; hymenium hyaline, inspersed with oil-droplets; asci 8-spored; ascospores hyaline to pale-brown, non-amyloid (I– or I+ wine red), muriform (9–16 × 1–3 locular), 50–75 × 9–15 µm.

Chemistry—Thallus K+ yellow, Pd+ orange, C–, KC–. Stictic acid detected with TLC.

Ecology—*Pallidogramme chrysenteron* was found growing on the bark of *Rhododendron* spp. (*Ericaceae*) above 2000 m in the Phulchowki forest area.

Specimen examined – **NEPAL. Bagmati zone:** Phulchowki, 27°34′41″N 85°23′40″E, alt. 2240 m, on tree bark, 04 February 2017, Neena Karmacharya 17–163B (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *P. chrysenteron*. The pantropical species has been recorded also from India, Sri Lanka, China, Brazil, Colombia, Costa Rica, Guyana, Panama, Australia, the Philippines, Thailand, and Vietnam (Staiger 2002, Singh & Sinha 2010, Schiefelbein & al. 2014, Joshi & al. 2015).

Pallidogramme divaricoides (Räsänen.) Pushpi Singh & Kr.P. Singh, Lichenologist 49: 531, 2017. Pl. 2h

Thallus corticolous, crustose, gray to greenish-gray, smooth, dull; photobiont *Trentepohlia;* ascomata lirellate, lirellae erumpent to prominent, curved to sinuous, elongate, divaricately branched, ≤10 mm long; disc concealed; labia striate; proper exciple pale-brown; hymenium hyaline, inspersed with oil-droplets; asci 8-spored; ascospores hyaline, amyloid (I+ blue), transversely 5–11-septate, 25–40 × 7–11 µm.

Chemistry—Thallus K–, Pd–, C–, KC–. No substances detected with TLC.

Ecology—*Pallidogramme divaricoides* was collected from the bark of *Alnus nepalensis* at c. 1600 m in the forest area of Phulchowki.

Specimen examined – **NEPAL. Bagmati zone:**, Phulchowki, 27°35′05″N 85°22′42″E, alt. 1626 m, on tree bark, 19 December 2016, Neena Karmacharya 16–089B (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *P. divaricoides*. It has been also reported from India (Singh & Singh 2017).

Phaeographis leiogrammodes (Kremp.) Müll. Arg., Nuovo Giorn. Bot. Ital. 23: 397, 1891. Pl. 2i

Thallus corticolous, crustose, pale to creamy-white, grayish, sometimes concolorous with substratum, smooth to verrucose, continuous; photobiont *Trentepohlia;* ascomata lirellate, lirellae immersed to level with thallus, numerous, simple, short to elongate, sparsely branched, stellate with a prominent thalline margin, 1–3 × 0.1–0.5 mm; disc exposed, concave to flattened, grayish, white pruinose; proper exciple thin, indistinctly present, hyaline to yellowish; hymenium hyaline, clear; asci 8-spored; ascospores brownish, amyloid (I– or I+ wine red), submuriform (8–14 × 1–2 locular µm), ≤40 × 9–15 µm.

Chemistry—Thallus K+ orange-red, Pd+ orange, C–, KC–. Norstictic and stictic acids detected with TLC.

Ecology—*Phaeographis leiogrammodes* was collected in association with other graphidoid species from the bark of *Castanopsis indica* at c. 1800 m from Shivapuri National Park in Kathmandu.

Specimen examined – **NEPAL. Bagmati zone:** Shivapuri National Park, 27°47′21″N 85°22′36″E, alt. 1802 m, on tree bark, 11 February 2017, Neena Karmacharya 17–196A (KATH).

Comments—Our Nepalese specimen corresponded closely to previously published descriptions of *P. leiogrammodes*. The pantropical species has been recorded also from China, Costa Rica, Thailand, Ecuador, and Netherlands (Staiger 2002, Aptroot & al. 2007, Bungartz & al. 2010).

Key to the species of *Graphidaceae* recorded from Nepal

1. Proper exciple hyaline to brown, not carbonized 2
1. Proper exciple distinctly carbonized, at least in some parts 7
2. Hymenium clear 3
2. Hymenium inspersed with oil-droplets 6
3. Disc concealed;
asci 2–4-spored; ascospores brownish, 28–60 × 10–17 µm;
norstictic acid .... ***Platythecium pyrrhochroum*** [≡ *Phaeographina pyrrhochroa*]
3. Disc broadly exposed 4
4. Asci 1-spored; ascospores muriform, hyaline, 95–150 × 30–40 µm;
stictic acid ..... ***Diorygma hieroglyphicum***
4. Asci 1–8-spored; ascospores sub-muriform to muriform, hyaline to brown;
norstictic and/or stictic acids 5
5. Asci 1–2-spored; ascospores muriform, hyaline, 84–134 × 29–42 µm,
norstictic acid ..... ***Diorygma junghuhnii***
5. Ascospores 8-spored; ascospores sub-muriform, brownish, ≤40 × 9–15 µm,
norstictic and stictic acids ..... ***Phaeographis leiogrammodes***
6. Lirellae prominent to erumpent, divaricately branched;
ascospores transversely septate, hyaline, 25–40 × 7–11 µm;
no substances ..... ***Pallidogramme divaricoides***
6. Lirellae sessile, irregularly branched, often aggregate;
ascospores muriform, hyaline to pale-brown, 50–75 × 9–15 µm;
stictic acid ..... ***Pallidogramme chrysenteron***
7. Ascomata embedded in distinctly raised structures (stromata)
on thallus; ascospores transversely septate, 28–60 × 7–10 µm; no
substances ..... ***Glyphis cicatricosa***
7. Ascomata not embedded in stromata, solitary to aggregate;
ascospores transversely septate to muriform; substances present or absent 8
8. Labia entire 9
8. Labia striate 16
9. Proper exciple laterally carbonized 10
9. Proper exciple completely carbonized 15

10. Hymenium clear 11
10. Hymenium inspersed with oil-droplets 13
11. Ascospores muriform, 20–40 × 8–12 µm; lirellae with apically thin, complete thalline margin; norstictic acid ..... ***Graphis pertricosa***
11. Ascospores transversely septate 12
12. Disc partly exposed, thinly white pruinose; lirellae with lateral to complete thalline margin; ascospores, 25–60 × 7–9 µm; no substances .... ***Graphis scripta***
12. Disc concealed; lirellae with lateral thalline margin; ascospores 25–35 × 10–12 µm; no substances ..... ***Graphis pinicola***
13. Ascospores muriform, 35–50 × 8–15 µm; lirellae with lateral thalline margin; no substances ..... ***Graphis subvelata***
13. Ascospores transversely septate 14
14. Norstictic acid; lirellae with basal to lateral thalline margin; ascospores 25–40 × 7–10 µm ..... ***Graphis cincta***
14. No substances; lirellae with lateral thalline margin; ascospores 20–40 × 6–9 µm ..... ***Graphis lineola***
15. Hymenium clear; lirellae sessile with thick lateral thalline margin; ascospores muriform, 85–120 × 15–20 µm ..... ***Graphis cleistoblephara***
15. Hymenium inspersed with oil-droplets; lirellae prominent with thick lateral thalline margin; ascospores muriform 60–110 × 15–30 µm .. ***Graphis leprographa***
16. Proper exciple apically (to peripherally) carbonized 17
16. Proper exciple laterally or completely carbonized 23
17. Ascospores transversely septate 18
17. Ascospores muriform 20
18. Stictic acid; lirellae lacking thalline margin; ascospores 20–35 × 6–9 µm ..... ***Graphis stenotera***
18. No substances 19
19. Lirellae with lateral thalline margin; ascospores 28–45 × 5–8 µm ..... ***Graphis chlorotica***
19. Lirellae lacking or with basal thalline margin; ascospores 20–48 × 5–8 µm ..... ***Graphis proserpens***
20. Norstictic or stictic acids 21
20. No substances 22
21. Norstictic acid; lirellae erumpent with lateral thalline margin; ascospores 30–60 × 17–25 µm ..... ***Graphis antillarum***
21. Stictic acid; lirellae erumpent to prominent with lateral thalline margin; ascospores 20–45 × 9–16 µm ..... ***Graphis galactoderma***
22. Lirellae radiately branched, lacking or with basal thalline margin; ascospores 25–40 × 8–15 µm ..... ***Graphis paradisserpens***
22. Lirellae irregularly branched with apically thin complete thalline margin; ascospores 30–40 × 9–12 µm ..... ***Graphis paraserpens***

23. Proper exciple laterally carbonized; lirellae with lateral thalline margin; ascospores transversely septate, 20–35 × 5–7 µm; no substances ..... ***Graphis tenella*** [= ***G. subglauconigra***]

23. Proper exciple completely carbonized; lirellae with apically thin complete thalline margin; ascospores muriform, 30–45 × 9–12 µm; norstictic acid ..... ***Graphis breussii***

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Sistotrema macrosporum sp. nov. from India

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Abstract—A new corticioid species, *Sistotrema macrosporum*, is described from Himachal Pradesh, India.

Key words—*Basidiomycota, Agaricomycetes,* Himalaya *Hydnaceae*

Introduction

During the fungal forays conducted in August 2012 in the Chaupal area of district Shimla, Himachal Pradesh, India, Maninder & Avneet collected a fungus on decaying wood of *Cedrus deodara* in a mixed coniferous forest. On the basis of comparison of macroscopic and microscopic characters (Eriksson & al. 1984, Gorjón & Hallenberg 2008, Dhingra & al. 2009, 2014; Bernicchia & Gorjón 2010, Prasher & Ashok 2013, MycoBank 2018) we referred the fungus to *Sistotrema* (*Hydnaceae*, *Agaricomycetes*, *Basidiomycota*). The specimens resembled *S. sernanderi* (Litsch.) Donk in basidiospore shape and the presence of gloeocystidia, but differed in its larger basidiospores. Here we describe the fungus as a new species, *S. macrosporum*.

Materials & methods

Samples were collected during field trips conducted in Chaupal forest division of district Shimla (Himachal Pradesh) during August 2012. Observations regarding locality/forest type, host/substrate, basidiocarp texture, hymenial surface colour and type, margins, etc. were recorded in the field. Colours are coded according to

Kornerup & Wanscher (1978). After removal of extraneous matter, the collected specimen was dried in sun and in a portable electric drier. The dried material was deposited at the Herbarium, Department of Botany, Punjabi University, Patiala, India (PUN). Crush mounts and free hand sections were used to study the micromorphological details (hyphae, cystidia, basidia, basidiospores, etc.) in water and 3%, 5%, and 10% KOH solutions and stained in cotton blue (1% in lactophenol), Congo red (1% in distilled water), phloxine (1% in distilled water), sulphovanillin (0.5gm vanillin + 4.0ml conc. sulphuric acid + 2.0ml distilled water), and Melzer's reagent (0.5gm iodine + 1.5gm KI + 20gm chloral hydrate + 20ml distilled water). Line diagrams of various microscopic structures were drawn using a camera lucida at 100×, 400×, and 1000× magnifications of the compound microscope. We then compiled data for identification and comparison with the published literature.

Taxonomy

Sistotrema macrosporum Man. Kaur, R. Kaur, Avn. P. Singh & Dhingra, **sp. nov.** PLATE 1

MYCOBANK 825447

Differs from *Sistotrema sernanderi* by its longer and wider basidiospores.

TYPE: India, Himachal Pradesh: Shimla, Chaupal, on the bark of decaying wood of *Cedrus deodara* (Roxb. ex D. Don) G. Don, 18 August 2012, Maninder Kaur 7920 (**Holotype**, PUN).

ETYMOLOGY: The epithet refers to the large basidiospore size.

Basidiocarps resupinate, effused, loosely adnate, ≤250 µm thick in section; hymenial surface smooth, tuberculate under lens, grayish white to light gray when fresh, brownish gray on drying; margins thinning, pruinose, paler concolorous, or indeterminate. Hyphal system monomitic. Generative hyphae ≤3.7 µm wide, septate, clamped, thin-walled, with oily contents; basal hyphae parallel to the substrate, less branched, with ampullate swellings; subhymenial hyphae vertical, more branched. Gloeocystidia 47–65 × 6–8 µm, flexuous, thin- to thick-walled, with basal clamp; oily contents negative to sulphovanillin. Basidia 20–34 × 8–9 µm, sub-urniform to urniform, 4-sterigmate, with basal clamp and oily contents; sterigmata ≤5.8 µm long. Basidiospores 8–11.5 × 3–3.7 µm, narrowly ellipsoid to suballantoid, thin-walled, smooth, with oily contents, acyanophilous, inamyloid.

Discussion

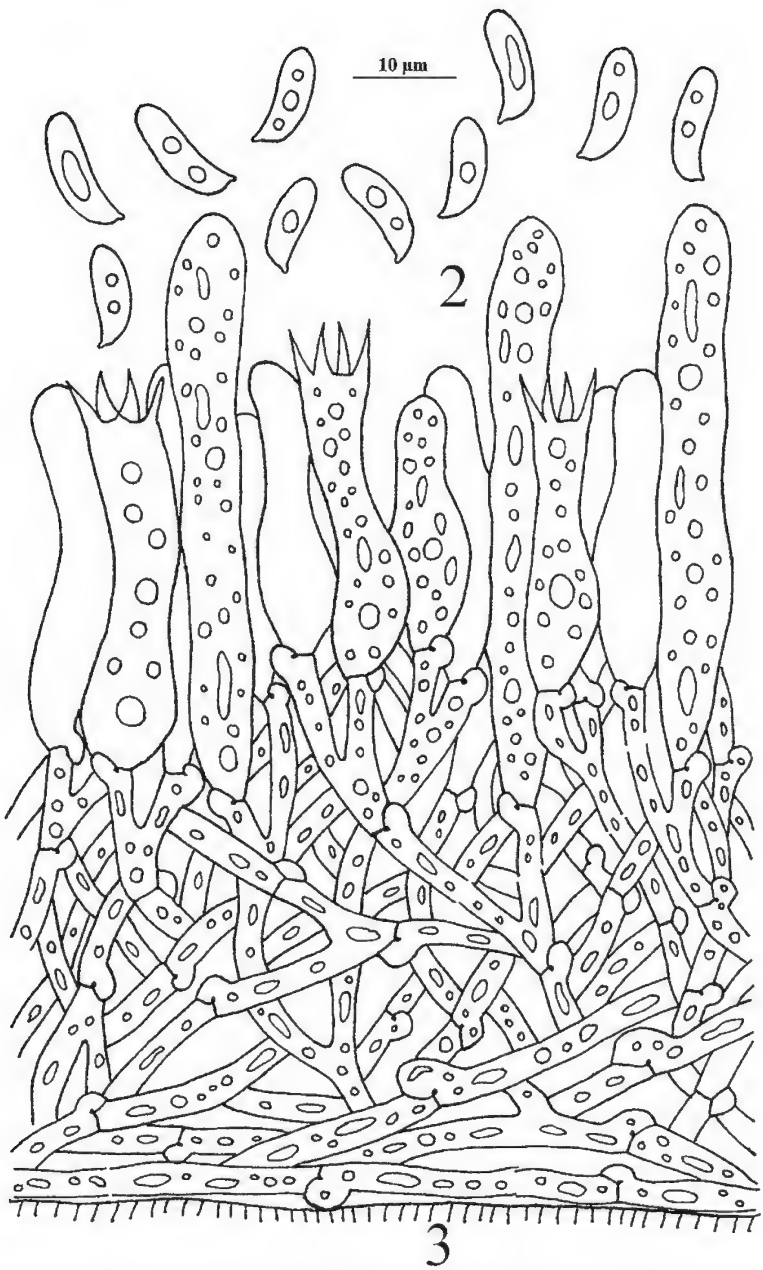
Thirteen *Sistotrema* species have previously been reported from India: *S. angustisporum* Dhingra, *S. biggsiae* Hallenb., *S. binucleosporum* Hallenb., *S. brinkmannii* (Bres.) J. Erikss., *S. confluens* Pers., *S. diademiferum* (Bourdot & Galzin) Donk, *S. heteronemum* (J. Erikss.) Å. Strid, *S. kirghizicum* (Parmasto)

Fig. 1. *Sistotrema macrosporum* (holotype, PUN (Kaur 7920). Basidiocarp showing hymenial surface. Scale = 5 mm.

Domański, *S. lachrymisporum* S.S. Rattan, *S. octosporum* (Höhn. & Litsch.) Hallenb., *S. porulosum* Hallenb., *S. sernanderi*, and *S. subtrigonospermum* D.P. Rogers (Rattan 1977; Dhingra & Singla 1997; Dhingra 2004; Dhingra & al. 2009; Sharma 2012, 2017; Samita 2014; Kaur & al. 2018). The new species *S. macrosporum* extends this total to fourteen.

A key to the *Sistotrema* species recorded from India

1. Cystidia absent 2
1. Cystidia present 3
2. Basidiospores ≤7 µm long ..... ***S. sernanderi***
2. Basidiospores >7 µm long ..... ***S. macrosporum***
3. Cystidia encrusted ..... ***S. angustisporum***
3. Cystidia not encrusted 4
4. Cystidia basally widened, without oily contents ..... ***S. kirghizicum***
4. Cystidia flexuous, with oily contents 5
5. Basidiospores tetrahedral ..... ***S. subtrigonospermum***
5. Basidiospores not pyramid-shaped 6
6. Basidiocarp stipitate ..... ***S. confluens***
6. Basidiocarp resupinate 7
7. Basidiospores allantoid 8
7. Basidiospores not sausage-shaped 9
8. Basidiocarp smooth to odontioid ..... ***S. brinkmannii***
8. Basidiocarp smooth to porulose ..... ***S. porulosum***
9. Basidiospores tear-drop shaped ..... ***S. lachrymisporum***
9. Basidiospores ellipsoid to cylindrical to ovate to oblong 10
10. Some basal hyphae light brown pigmented ..... ***S. heteronemum***
10. All hyphae hyaline 11



Figs 2,3. *Sistotrema macrosporum* (holotype, PUN (Kaur 7920).
2. Basidiospores; 3. Vertical section through basidiocarp.

11. Basidiocarp membranaceous, with loose texture 12
11. Basidiocarp thin, adnate, porulose 13
12. Basidiocarp fibrillose, with strands ..... ***S. octosporum***
12. Basidiocarp smooth, without strands ..... ***S. biggsiae***
13. Basidiospores ellipsoid to ovoid-subglobose, 3.5–5 × 3–3.5 µm ..... ***S. diademiferum***
13. Basidiospores subcylindrical to ellipsoid, 3.3–3.8 × 1.3–1.8 µm ..... ***S. binucleosporum***

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A preliminary study of the yellow *Acarospora* of China

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Abstract—The four species of yellow *Acarospora* recorded from China (*A. fulva, A heufleriana, A. schleicheri*, and *A. stapfiana*) are described in detail from specimens collected in the Xinjiang region of central Asia. *Acarospora fulva* and *A. stapfiana* are reported as new for China. A key to species of yellow *Acarospora* in China is provided. Sequences of three species from this study have been deposited in GenBank.

Key words—*Acarosporaceae,* biodiversity, rhizocarpic acid, taxonomy

Introduction

In *Lecanoromycetes* O.E. Erikss. & Winka, Subclass *Acarosporomycetidae* V. Reeb & al. was proposed by Reeb & al. (2004) for a single order *Acarosporales* V. Reeb & al. and containing a single family of lichenized and non-lichenized lichenicolous fungi, *Acarosporaceae* Zahlbr. (Jaklitsch & al. 2016, Miadlikowska & al. 2014). *Acarosporomycetidae,* recognized as one of the oldest lichenized fungal taxa, are thought to have diverged from other lecanoromyceteous lineages during the Carboniferous period 299–365 million years ago (Prieto & Wedin 2013). Its species characterized by polyspored asci and a green alga photobiont, are crustose lichens that occur on rock and soil, often as pioneers and usually in arid habitats (Magnusson 1929). The family currently contains over 400 taxa, but due to taxonomic and nomenclatural problems and the lack of a modern global revision, a calculation of the exact number of species is not

possible (http://www.indexfungorum.org). Seven genera are now represented: *Acarospora* A. Massal., *Glypholecia* Nyl., *Myriospora* Nägeli ex Uloth, *Pleopsidium* Körb., *Sarcogyne* Flot., *Timdalia* Hafellner, and *Trimmathelopsis* Zschacke (Arcadia & Knudsen 2012, Knudsen & Lendemer 2016, Westberg & al. 2015). The genera *Polysporina* Vězda and *Thelocarpella* Nav.-Ros. & Cl. Roux were found to be paraphyletic, and their taxa have been transferred to other genera (Knudsen & Lendemer 2016, Westberg & al. 2015).

Yellow species of *Acarospora, Pleopsidium,* and *Sarcogyne* have been reported from around the world (Costello & Nimis 1994, Hafellner 1993, Knudsen 2007, McCarthy & Elix 2017, Magnusson 1929, 1956). One hundred and two species of *Acarosporaceae* have a yellow cortex (Costello & Nimis 1994; Golubkova 1979; Knudsen 2007; Knudsen & al. 2008; Knudsen & Elix 2008; Knudsen & Flakus 2009, 2016; Lumbsch & al. 2011; McCarthy & Elix 2017; Magnusson 1929, 1956; Øvstedal & al. 1918; Roux & Wirth 2011). The yellow color is from rhizocarpic acid and/or epanorin. Epanorin occasionally occurs with rhizocarpic acid in major or minor concentrations in some species or may replace rhizocarpic acid (e.g., in *A. congregata* K. Knudsen & Flakus in South America; Knudsen & al. 2008, Knudsen & Flakus 2016). Both secondary metabolites are derived from pulvinic acid as are calycin and pinastric acid commonly found in yellow species of *Chrysotrix* and *Candelariella* (Huneck & Yoshimura 1996). Rhizocarpic acid allows growth of yellow *Acarosporaceae* in dry habitats with high sunlight and extreme temperatures and on nutrient poor acidic or calcareous substrates in deserts and mountains as well as south slopes in temperate habitats (Hauck & al. 2010, Meeßen & al. 2013, Rubio & al. 2002).

Magnusson (1929) proposed *Acarospora* subg. *Xanthothallia* H. Magn. for yellow *Acarospora,* but molecular phylogenies do not support yellow species within a monophyletic group (Leavitt & al. 1918, Reeb & al. 2004, Westberg & al. 2015). *Acarospora* subg. *Xanthothallia* is considered an artificial taxon and no longer accepted.

Studying collections of *Acarosporaceae* from Gansu in China, Magnusson reported one yellow species, *Pleopsidium gobiense* (H. Magn.) Hafellner (Magnusson 1940; as *Acarospora gobiensis* H. Magn.). Magnusson reported five yellow species from Southern Mongolia: *P. discurrens* (Zahlbr.) Obermayer (as *A. discurrens*), *P. gobiense, A. heufleriana, A.* cf. *congregata* (as *Acarospora* cf. *xanthophana*), and *A. schleicheri* (Magnusson 1944). Russian lichenologist Golubkova (1988) reported seven species from central Asia in U.S.S.R.: *Pleopsidium chlorophanum* (Wahlenb.) Zopf (as *A. chlorophana*), *P. flavum* (Bellardi) Körb. (as *Acarospora oxytona*), *P. gobiense, A. heufleriana,*

A. schleicheri, A. stapfiana, and *A. sulphurata* (Arnold) Arnold. *Pleopsidium discurrens* was reported from Tibet (Obermayer 1996), and additional records of *P. chlorophanum, P. discurrens, P. flavum,* and *P. gobiense* were reported from China by Niu & al. (2015, 2017).

Eight yellow representatives of *Acarosporaceae* occur in China: four *Acarospora* species and four *Pleopsidium* species. In this paper we report four yellow *Acarospora* species for China from the Xinjiang region of central Asia: *A. heufleriana* and *A. schleicheri* (Wei 1991) as well as *A. fulva* and *A. stapfiana,* which are reported here from China for the first time.

Material & methods

The examined specimens are preserved in Arid Land Lichen Research Center of Western China, Xinjiang University, Urumqi, China (XJU-NALH). They were morphologically examined with Olympus STM7 microscope and photographed with Nikon DS-Fi2 and Canon PC1200 cameras. Measurements were made in water and the hymenial gel was tested for amyloidity with Lugol's (IKI). Spot tests were made for norstictic acid with potassium hydroxide (KOH) and for gyrophoric acid with potassium hydroxide and sodium hypochlorite (KC). Sequences from specimens in this study representing *A. fulva, A heufleriana,* and *A. stapfiana* were deposited in GenBank and used by Øvstedal & al. (2018) in their phylogeny of yellow *Acarospora*.

Taxonomy

Acarospora fulva N.S. Golubk., Nov. Sist. Niz. Rast. 16: 130 (1979). Fig. 1e, f

Thallus areolate, contiguous, at first flat then convex, rugulose, with deep cracks between areoles, round to irregular; areoles eventually forming stipes and becoming squamulose, 0.5–3 mm wide, 0.4–0.8 mm high, rim downturned, sometimes blackish or concolorous with upper surface. Upper surface yellow, becoming greenish yellow when wet, fissured, epruinose; lower surface corticated, and yellow, brownish yellow, or blackish. Cortex 40–65 µm thick, paraplectenchymatous to prosoplectenchymatous, cells varying in size. Algal layer uneven, interrupted by hyphal bundles, ≤150 µm thick, algal cells 5–14 µm in diam. Medulla white, prosoplectenchymatous, ≤450 µm thick.

Apothecia immersed, punctiform at first, then expanded, usually one per areole, varying in shape, 0.3–1.2 mm wide, disc blackish brown, sometimes with a yellow umbo, rough, fissured. Parathecium hyaline, distinct, 15–25 µm wide, expanded, ≤75 µm around the disc. Epihymenium yellowish brown, 10–20 µm tall. Hymenium hyaline, interascal gel I+blue turning red (hemiamyloid), 50–85 µm tall, paraphyses 2–3 µm diam, apices brownish black, expanding to 4 µm diam. Asci clavate, 40–72 × 6–15 µm, with about 100 ascospores,

ascospores various in size, hyaline, simple, 3.5–6 × 1.8–3 µm. Subhymenium 30–50 µm tall. Hypothecium 35–65 µm tall.

Spot tests: cortex C+ red, KC+ red. Secondary metabolites: gyrophoric acid, rhizocarpic acid. ITS sequences: MF134868, MF134867.

Selected specimens examined: **CHINA. Inner Mongolia. Baotou**, alt. 1535 m, 6 August 2014, A. Abbas, G. Sahedat, L. Nurtai 20141065a, 20141065b, 20141062b (GenBank MF134867), 20141080, 20141058 (XJU-NALH). **Xinjiang. Altay Mountain**, alt. 1075 m, 5 May 2014, A. Abbas 2014050a, 2014051a, 2014052a (XJU-NALH); **Qinghe**, alt. 1299 m, 17 June 2013, A. Abbas 20130706a (XJU-NALH); alt. 1294 m, 17 June 2013, A. Abbas 20130707b, 201306af (XJU-NALH); alt. 1290 m, 17 June 2013, A. Abbas 20130627, 20130808 (XJU-NALH); **Turhong Township**, alt. 1470 m, 21 July 2012, A. Abbas 20122442, 20122500 (XJU-NALH).

Ecology & distribution. Occurs on siliceous rock. Endemic to central Asia (China, Mongolia); this is a new species record for China.

Discussion. The species is easily identified with spot tests. Only four other yellow *Acarospora* species with gyrophoric acid have been described: *A. ramosa* K. Knudsen & Flakus from South America (Knudsen & Flakus 2009), and *A. erythrophora* H. Magn., *A. robiniae* K. Knudsen, and *A. tuckerae* K. Knudsen from North America (Knudsen 2007). *Acarospora fulva* has a lower hymenium than *A. erythrophora* (100–170 µm; Knudsen 2007); a lower hymenium and thinner upper cortex than *A. robiniae* (hymenium, 80–120 µm; cortex, 80–100 µm; Knudsen 2007); and a lower hymenium than *A. ramosa* (150–180 µm; Knudsen & Flakus 2009). The algal layer of *A. tuckerae* is even and only the medulla is C+ red with gyrophoric acid, whereas that of *A. fulva* is jagged and interrupted by hyphal bundles, and the cortex is C+ red with gyrophoric acid (Knudsen 2007). Golubkova (1988) referred the earlier report of *A.* cf. *congregata* (Magnusson 1944, as *A.* cf. *xanthophana*) to *A. fulva*.

Acarospora heufleriana Körb., Parerga Lichenol. 1: 57 (1859). Fig. 1a–d

Thallus areolate, contiguous to dispersed, flat, broadly attached to the substratum, areoles angular, 0.3–1.5 mm wide, 250–320 µm high. Upper surface greenish yellow to yellow, yellowish gray when pruinose, usually smooth and glossy when epruinose, or cracking. Cortex 50–90 µm thick, with round cells, the cells 4–7 µm in diam. Algal layer even to uneven, sometimes interrupted by hyphal bundles, cells 7.5–17 µm in diam. Medulla white, prosoplectenchymatous, hyphae anti-clinal, ≤250 µm thick.

Apothecia 1–3 per areoles, smooth immersed, round or irregular, disc dark brown to brown or yellowish, rough, epruinose. Parathecium 25–45 µm wide. Epihymenium yellow, 5–10 µm tall. Hymenium hyaline to yellowish,

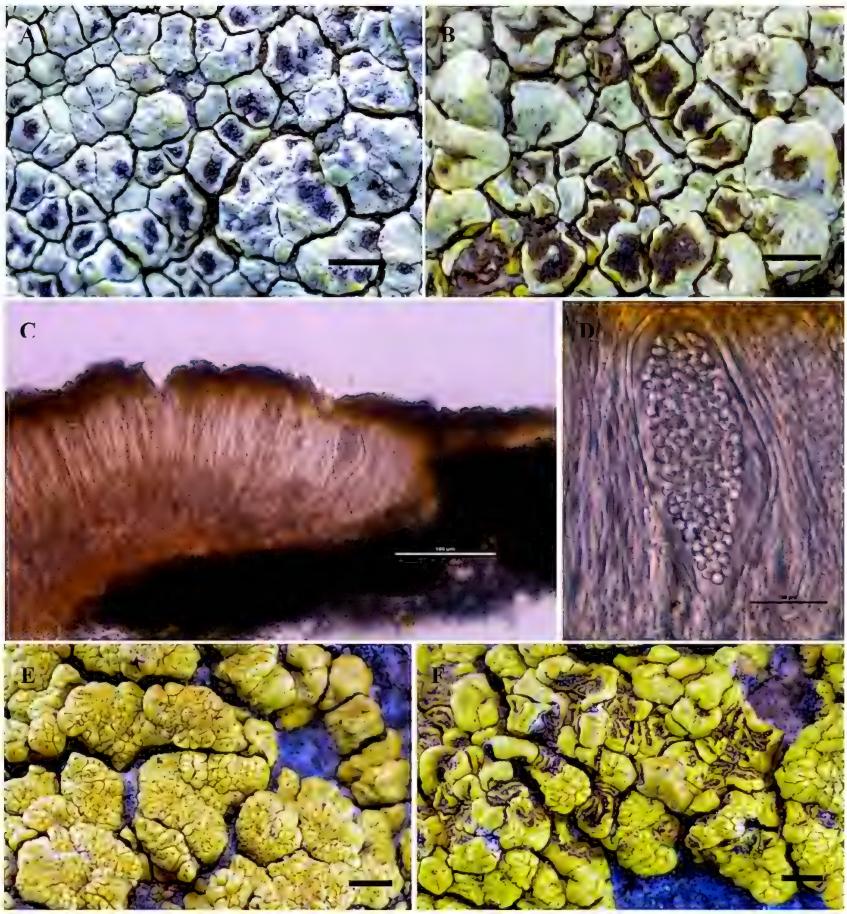

FIG. 1. ***Acarospora heufleriana*** (XJU-NALH.20141106): A, B. Thallus and apothecia; C, D. Cross section of apothecia and ascus. ***Acarospora fulva*** (XJU-NALH.2014050a): E, F. Thallus and apothecia. Scale bars: A, B, E, F = 1 mm; C = 100 μm; D = 20 μm.

interascal gel I+ greenish blue turning red (hemiamyloid), 92–120 μm tall. Asci clavate, with 100–200 ascospores, 81–110 × 17–28 μm; ascospores hyaline, simple, ellipsoid, 3.5–5.0 × 2–2.8 μm. Subhymenium 30–40 μm tall, I+ blue, hypothecium yellowish, 25–45 μm tall.

SPOT TEST: medulla K+ red (forming distinct crystals). SECONDARY METABOLITES: rhizocarpic acid, norstictic acid. ITS SEQUENCE: MF134874.

SPECIMENS EXAMINED: **CHINA. INNER MONGOLIA. Daqingshan**, alt. 1255 m, 1 August 2014, A. Abbas, G. Sahedat, L. Nurtai 20141105a, 20141105b, 20141105c, 20141130b,

20141090b, 20141090c, 20141107, 20141108, 20141090a, 20141106, 20141130a (XJU-NALH); alt. 1719 m, 1 August 2014, A. Abbas, G. Sahedat, L. Nurtai 20141147 (XJU-NALH); **Mount Huaguo**, alt. 1535 m, 6 August 2014, A. Abbas, G. Sahedat, L. Nurtai 20141072b, 20141072a (GenBank MF134874), 20141075 (XJU-NALH).

Ecology & distribution. On siliceous rock. Asia (Magnusson 1944, Golubkova 1988), Europe (Magnusson 1929), North America (Knudsen 2007).

Discussion. *Acarospora heufleriana* produces the secondary metabolite norstictic acid and is easily identified with spot tests. Two other yellow *Acarospora* species with norstictic acid have been reported from North America (Knudsen 2007). *Acarospora calcarea* K. Knudsen differs from *A. heufleriana* primarily in being effigurate (not effuse), and *A. rouxii* K. Knudsen & al. is distinguished by its higher (100–170 µm) hymenium.

Acarospora schleicheri (Ach.) A. Massal.,

Ric. Auton. Lich. Crost.: 27 (1852). Fig. 2 c, d, g

Thallus areolate to squamulose, contiguous, broadly attached to the substratum by rhizohyphae, upper surface pruinose to epruinose, rough or smooth, dull, squamules 0.3–2.7(–3.2) mm wide, ≤270 µm thick. Cortex 40–80 µm thick, paraplectenchymatous to prosoplectenchymatous. Algal layer uneven, interrupted by hyphal bundles, ≤130 µm thick. Medulla white, prosoplectenchymatous, hypha thin-walled, ≤200 µm thick.

Apothecia immersed, round to irregular, disc blackish brown or brown or yellowish, rough, pruinose to epruinose. Parathecium indistinct. Epihymenium yellowish brown, 10–18 µm tall. Hymenium hyaline, with crystals, I+ blue (amyloid), 80–130 µm tall, paraphyses 1–2.3 µm diam, apices expanded ≤5 µm. Asci clavate, with 100+ ascospores; ascospores broadly ellipsoid or subglobose, with one or two oil spots, hyaline, simple, 3–7 × 2–4.5 µm. Subhymenium hyaline, 30–55 µm. Hypothecium indistinct.

Spot tests: negative. Secondary metabolites: rhizocarpic acid.

Selected specimens examined: **China. Tibet. Lasa**, alt. 4731 m, 24 September 2012, Wen 20120924 (XJU-NALH); alt. 4960 m, 24 September 2012, Wen 20120925 (XJU-NALH); alt. 4432 m, 10 September 2011, Wen 20110910 (XJU-NALH).

Ecology & distribution. *Acarospora schleicheri* is quite common in the Northern Hemisphere, reported from central Asia (Abbas & Wu 1998, Golubkova 1988, Magnusson 1944), Europe, North Africa, and North America (Magnusson 1929).

Discussion. *Acarospora schleicheri* is an obligate terricolous lichen with distinct rhizohyphae that bind and hold together soil crusts. It occurs on both

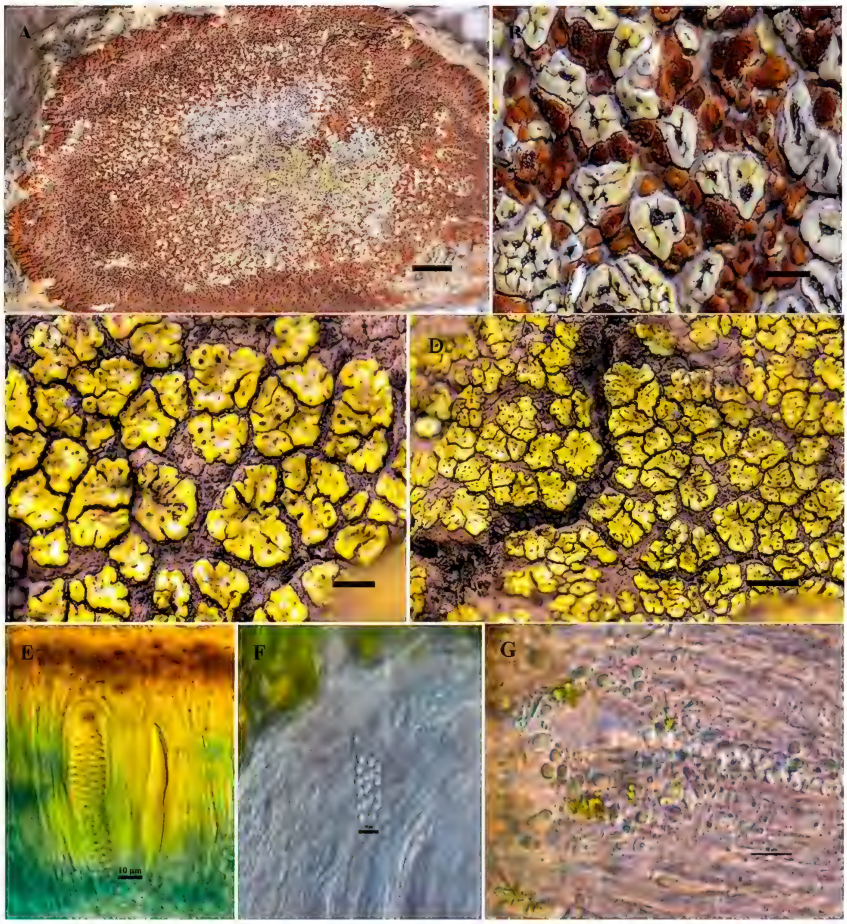

FIG. 2. ***Acarospora stapfiana*** (XJU-NALH 20110389): A, B. Thallus and apothecia; E, F. Ascus after iodine reaction and spherical ascospores. ***Acarospora schleicheri*** (XJU-NALH 20120924): C, D. Thallus and apothecia; G. Ascospores. Scale bars: A–D = 1 mm; E–G = 10 µm

calcareous and non-calcareous soils and spreads both through division of squamules and through spreading rhizohyphae, which form new squamules sometimes several centimeters from main thallus. It has been reported as a parasite on other lichens; its rhizohyphae penetrate other lichens (such as *Diploschistes muscorum* (Scop.) R. Sant.) in soil crusts from below and destroy them as it forms new squamules. It is not an obligate parasite but an opportunistic parasite in competition for space and may not even steal the algae of the host, as probably is the case in most lichenicolous lichens.

Acarospora stapfiana (Müll. Arg.) Hue,
Nouv. Arch. Mus. Hist. Nat., Paris, 5 sér., 1: 161 (1909). FIG. 2A, B, E, F

THALLUS areolate to subsquamulose, contiguous, little dispersed, parasitic on *Caloplaca* species, areoles round. Upper surface greenish yellow to yellow, or gray when pruinose, smooth to cracked, 0.3–1.7 mm wide, ≤900 µm high. Cortex 45–70 µm thick, paraplectenchymatous to prosoplectenchymatous. Algal layer even, ≤205 µm thick, cells 5–15 µm diam. Medulla white, prosoplectenchymatous, ≤380 µm thick.

APOTHECIA 1–3 per areole, with radial fissures around apothecia, punctiform at first, then immersed, round, disc reddish brown, rough, epruinose. Parathecium 8–30 µm wide. Epihymenium reddish brown, 15–25 µm tall. Hymenium hyaline, 90–110 µm tall, I+ dark blue, paraphyses 1.5–2.5 µm diam, apices expanded and brown, ≤4.5 µm. Asci clavate, with 100+ ascospores, 56–65 × 17–20 µm; ascospores globose or broadly ellipsoid, hyaline, simple, 3–5 × 3–4 µm Subhymenium indistinct 20–35 µm tall, hypothecium hyaline, 15–25 µm tall.

SPOT TEST: negative. SECONDARY METABOLITES: rhizocarpic acid. ITS SEQUENCES: MF134871, MF134870.

SPECIMENS EXAMINED: **CHINA. XINJIANG. Aktau**, alt. 4320 m, 1 August 2011, A. Abbas 20110389 (XJU-NALH; GenBank MF134870): **Shirengou**, alt. 1543 m, 25 November 2013, A. Abbas, L. Nurtai, G. Nazarbek 20130040 (XJU-NALH; GenBank MF134871); **Wucaiwan**, alt. 2070 m, 4 June 2011, A. Abbas AS110604 (XJU-NALH); **Altin Mountain**, alt. 4000 m, 14 July 2005, A. Abbas 500110 (XJU-NALH).

ECOLOGY & DISTRIBUTION. Usually on calcareous rock, parasitic on orange saxicolous *Caloplaca* species in Asia (Golubkova 1988, Magnusson 1929, 1944) and North America (Knudsen 2007); this is a new record for China.

DISCUSSION. *Acarospora stapfiana* is a lichenicolous lichen and obligate parasite. It begins its life cycle as a juvenile non-lichenized fungus within various *Caloplaca* species. It destroys the orange host, probably stealing its algal photobiont, and progressively morphs out of the host forming its own yellow lichenized thallus (Knudsen 2007).

A key to yellow *Acarospora* in China

1. Ascus tholus with I+ blue cylinder around ocular chamber (Hafellner 1993, Obermayer 1996) ..... [*Pleopsidium*]
[See Niu & al. 2017 for descriptions and key to Chinese material.]

1. Ascus tholus I– 2

2. On soil ..... *A. schleicheri*

2. On rock 3

3. Cortex C+ red, thallus usually forming a stipe ..... *A. fulva*
3. Cortex and medulla C– 4
4. Medulla K+ red, not parasitic on *Caloplaca* species ..... *A. heufleriana*
4. Medulla K–, parasitic on *Caloplaca* species ..... *A. stapfiana*

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Validation of five corticioid species from Eastern Himalaya

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Abstract—*Candelabrochaete himalayana*, *Conohypha grandispora*, *Fibulomyces cystoideus*, *Paullicorticium indicum,* and *Sistotrema angustisporum,* previously invalidly published because their type specimens were each deposited in two herbariums, are validated here by differentiation of the two depositions as holotype and isotype.

Key words—*Atheliaceae*, *Hydnaceae*, *Meruliaceae*, *Phanerochaetaceae*, *Polyporaceae*

Introduction

Of the six new species of corticioid fungi Dhingra (2004) described from the Eastern Himalaya, five names—*Candelabrochaete himalayana*, *Conohypha grandispora*, *Fibulomyces cystoideus*, *Paullicorticium indicum,* and *Sistotrema angustisporum*—were invalid because their protologues cited two herbaria for type specimens without designating a single herbarium as the holotype depository (Turland & al. 2018: Art. 40.7). Only one herbarium was indicated for the sixth species, *Leucogyrophana thimphina*, which was thus validly published in Dhingra (2004). The five invalid names are validated here.

Taxonomy

Candelabrochaete himalayana Dhingra, **sp. nov.**

Index Fungorum IF555616

"*Candelabrochaete himalayana*" Dhingra, Pl. Divers. India: 477. 2004, nom. inval. (ICN Art. 40.7) [IF632498].

Type: Bhutan, Thimphu, Paro, D'Dzong, on gymnosperm wood, 28 September 1980, G.S. Dhingra 19430 (**Holotype**, PAN; isotype, LY).

Description & illustrations: Dhingra (2004: 477–478, Figs 1–6).

Conohypha grandispora Dhingra, **sp. nov.**

Index Fungorum IF555617

"*Conohypha grandispora*" Dhingra, Pl. Divers. India: 478. 2004, (nom. inval. ICN Art. 40.7) [IF632499].

Type: India, West Bengal, about 7 km from Ghoom in Sukhia, on rotting trunk, 30 August 1980, G.S. Dhingra 19286 (**Holotype**, PAN; isotype, GH).

Description & illustrations: Dhingra (2004: 478–480, Figs 7–10).

Fibulomyces cystoideus Dhingra, **sp. nov.**

Index Fungorum IF555618

"*Fibulomyces cystoideus*" Dhingra, Pl. Divers. India: 480. 2004, (nom. inval. ICN Art. 40.7) [IF632500].

Type: Bhutan, Thimphu, Nawephu, on decaying *Pinus* wood, 18 September 1980, G.S. Dhingra 19365 (**Holotype**, PAN; isotype, O).

Description & illustrations: Dhingra (2004: 480–481, Figs 11–15).

Paullicorticium indicum Dhingra, **sp. nov.**

Index Fungorum IF555619

"*Paullicorticium indicum*" Dhingra, Pl. Divers. India: 483. 2004, (nom. inval. ICN Art. 40.7) [IF632502].

Type: India, West Bengal, Darjeeling, Tiger Hill, on rotting angiosperm trunk, 19 August 1980, G.S. Dhingra 19255 (**Holotype**, PAN; isotype, GH).

Description & illustrations: Dhingra (2004: 483, Figs 20–24).

Sistotrema angustisporum Dhingra, **sp. nov.**

Index Fungorum IF555620

"*Sistotrema angustispora*" Dhingra, Pl. Divers. India: 484. 2004, (nom. inval. ICN Art. 40.7) [IF632504].

Type: India, West Bengal, Darjeeling, about 5 km from Takdah on 6th Mile–Takdah road, 17 August 1980, G.S. Dhingra 19233 (**Holotype**, PAN; isotype, GH).

Description & illustrations: Dhingra (2004: 484, Figs 25–30).

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The authors thank Dr. Shaun Pennycook and Dr. Paul Kirk for the valuable suggestions regarding validity and correction of the species described earlier and Head, Department of Botany, Punjabi University, Patiala for necessary facilities. Mycotaxon Editors Pennycook and Dr. Lorelei Norvell provided presubmission reviews.

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New additions to Turkish macrofungi from Tokat and Yozgat Provinces

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Abstract—Macrofungal samples collected from Tokat and Yozgat provinces during 2014–2017 were identified as new records for the Turkish mycota: *Ciboria viridifusca, Clitocybe amarescens*, *Mycena polyadelpha*, *Rutstroemia conformata,* and *Symphyosirinia galii*. Short descriptions, illustrations, and discussions are provided.

Key words—*Agaricales,* biodiversity, Funga, *Helotiales,* taxonomy

Introduction

Checklists on Turkish larger fungi have been prepared by Sesli & Denchev (2014) and Solak & al. (2015). However, some new records have been added to the Turkish mycota by various studies (Akata & Doğan 2015, Doğan & Öztürk 2015, Kaya & Uzun 2015, Kaya 2015, Sesli & al. 2015, 2016; Sesli & Moreau 2015, Taşkın & al. 2016, Allı & al. 2017, Akata & Sesli 2017, Sesli & Vizzini 2017, Türkekul 2017, Işık & Türkekul 2017, 2018; Uzun & al. 2017, Uzun & Demirel 2017, Demirel & al. 2017, Akata & Gürkanlı 2018, Sesli 2018, Uzun & Kaya 2018, Kaya & al. 2018).

Tokat is situated in the inland part of the Middle Black Sea Region (Turkey); Tokat province lies about 650–1500 m asl and has a semi-arid climate. Field studies were carried out in areas which are generally surrounded by evergreen and deciduous forests. Akbelen highland (~30 km from Tokat city center at an average 1400 m elevation) is a rugged area surrounded by mixed

forests dominated by *Alnus glutinosa, Carpinus orientalis, Fagus orientalis, Quercus pubescens, Q. robur, Pinus nigra,* and *Populus alba*. Çamaltı village (~15 km from Tokat city center at an average 900 m elevation) is covered with similar vegetation but has a drier climate. The dominant plant in the region is *Quercus pubescens.*

In Yozgat province, Akdağmadeni district (at 1352 m asl with an average annual 480–500 mm precipitation and 8–12°C temperature) has a transition climate between Black Sea and continental climates. The eastern and southern parts of the province are covered with pine forests, predominantly *Pinus sylvestris,* in association with *P. nigra*, *Populus alba*, and species of *Juniperus*, *Salix*, *Crataegus*, *Pyrus*, *Quercus*, *Rosa*, and *Corylus* [https://www.akdagmadeni.bel.tr/akdagmadeni-ormanlari-s61.html (accessed 10 April 2018)].

The aim of the current study is to contribute to Turkish mycobiota.

Materials & methods

The fungal specimens were collected during field trips within Tokat province and Akdağmadeni (Yozgat) district during 2014-2017. The specimens were photographed in their natural habitats, their ecological features and macroscopic features noted, assigned collection numbers, transported to the laboratory in aluminium foil and boxes, and dried for later identification. Microscopic observations were made from dried herbarium specimens mounted in distilled water, Melzer's reagent, Congo red, or KOH using a light microscope. The specimens were identified based on macroscopic and microscopic characters after consulting Maas Geesteranus (1954), Dennis (1978), Ellis (1956, 1980), Moser (1983), Bon (1987), Breitenbach & Kränzlin (1984, 1991), Verkley (1993), Baral (1994), Palmer &Tortic (1994), Bas & al. (1995), Garcia & Moreau (2002), Gerault (2005), Hairaud& Capoen (2012), Alcántara & Mora (2013). The systematics of the newly recorded taxa are in accordance with Index Fungorum (http://www.indexfungorum.org: accessed 10 April 2018). The identified specimens were deposited in the Fungarium of Biology Department, Gaziosmanpaşa University, Tokat, Turkey (FGOU).

Taxonomy

Sclerotiniaceae

Ciboria viridifusca (Fuckel) Höhn.,
Mitt. Bot. Inst. T.H. Wien 3(3): 101 (1926) Fig. 1

Apothecia 1–4 mm, light brown and brown; cup-shaped to flat or convex atop a 1–2 mm long stalk; several fruiting bodies usually on one cone. Outer and inner surface of hymenium and stalk nearly concolorous, finely farinose. Ectal excipulum textura globulosa, medullary excipulum textura intricata.

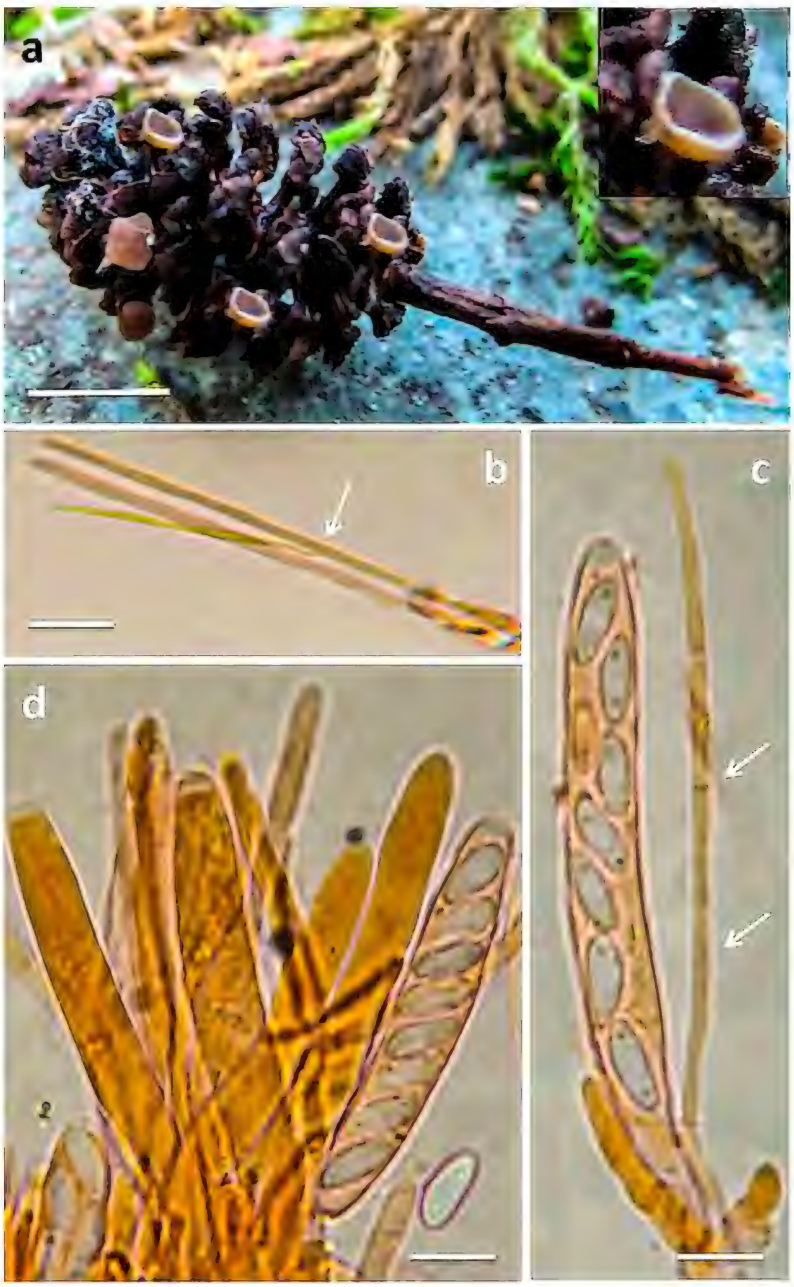


Fig. 1. *Ciboria viridifusca* (FGOU 5416): A. apothecia; B. forked paraphyses; C. amyloid apex of asci and ascospores; D. asci with ascospores and paraphyses. Scale bars: A = 3 mm; B–D = 10 μm.

Ascospores narrowly elliptical, hyaloid, smooth, without drops or with small drops at each pole, (7–)7.5–9.5(–11.3) × (3.2–)3.5–4.5(–5) µm. Asci (59.5–)60–80.2(–85.3) × (4.2–)5–9(–9.5) µm, cylindrical, 8-spored, inoperculate, spores monoseriate or irregularly biseriate. Paraphyses 60–80 × (2–)2.5–3(–4.1) µm, filiform, branched, non-septate or septate, sometimes with slight clavate swellings towards the tips.

Specimen examined: **TURKEY, Tokat, Akbelen village,** 40°27′56″N 36°38′33″E, 1077 m, on damp female cones of *Alnus glutinosa* (L.) Gaertn. (*Betulaceae*) 19 November 2017, leg. Türkekul & Işik (FGOU 5416).

Comments—Some ascospores of our Turkish specimen were bigger than those of relevant literature descriptions (Breitenbach & Kränzlin 1984: 6–7 × 2–2.5 µm; Dennis 1978: 6–9 × 3–4µm; Alcántara & Mora 2013: (5.8–)6.8–8.0 (–9.6) × (2.9–)3.3–3.8(–4.2) µm).

Several other *Ciboria* species also grow on fallen *Alnus* male catkins or female cones. *Ciboria amentacea* (Balb.) Fuckel resembles *C. viridifusca* but differs by growing on damp male *Alnus* catkins in spring. Another similar species is *Mollisia amenticola* (Sacc.) Rehm, which is distinguished by sessile, whitish, grayish, or light cream apothecia, and smaller spores (5–5.5 × 2–2.5 µm; Breitenbach & Kränzlin 1984, Alcántara & Mora 2013).

Tricholomataceae

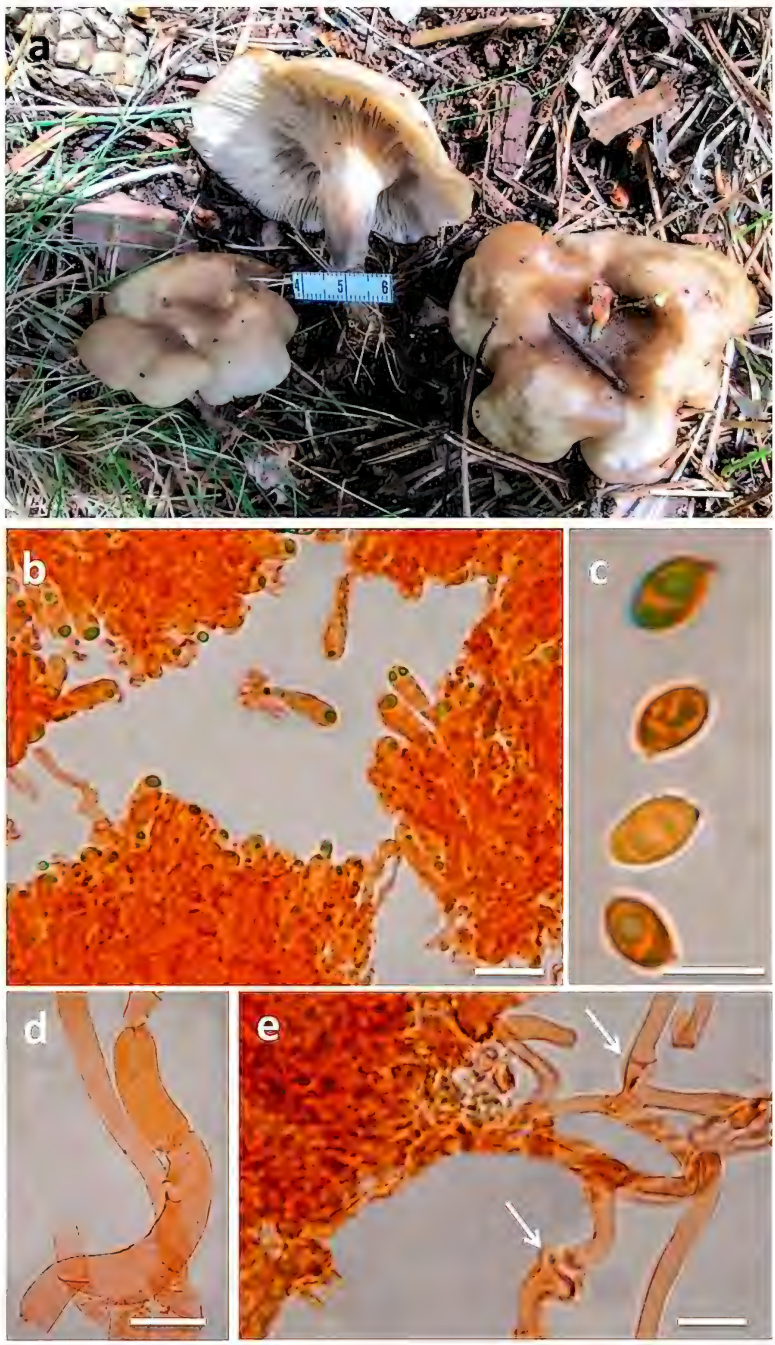
Clitocybe amarescens Harmaja, Karstenia 10: 98 (1969) Fig. 2

Pileus 3–6 cm, convex-depressed, grey or ochraceous brown, margin usually slightly striate, but sometimes smooth and often lobed, hygrophanous. Lamellae decurrent, close, cream-coloured or pale brown, slightly sloping. Stipe (20–)40 × 3–4(–6) mm, central, cylindrical to tapering downwards, concolorous with pileus with a darker base. Spore print pale cream.

Basidiospores (4.7–)5.0–9.0(–9.8) × (3.0–)3.5–5.0(–6.3) µm, elliptical, smooth. Basidia clavate, (19.3–)23–28(–28.9) × (4.6–)5–8(–8.6) µm, 4-spored. Cheilocystidia absent. Clamp connections present.

Specimen examined: **TURKEY, Yozgat–Akdağmadeni, Davulbazvillage,** 39°36′07″N 35°52′45″E, 1629 m, among needle and cone litters *Pinus sylvestris* L. (*Pinaceae*) forest, 17 November 2014, leg. Türkekul & Işik (FGOU 4406).

Fig. 2. *Clitocybe amarescens* (FGOU 4406): A. Basidiomata in situ; B. basidia; C. basidiospores; D. elements of pileipellis; E. Clamps on basidial bases. Scale bars: A = 2 cm; B, D = 20 µm; C, E = 10 µm.


a
b
c
d
e

Comments—Our Turkish specimen was morphologically and ecologically very similar to published descriptions of *Clitocybe amarescens* (Bon 1987, Moser 1993, Bas & al. 1995).

Clitocybe amarescens is close to *C. metachroa* (Fr.) P. Kumm. and *C. stercoraria* H.E. Bigelow. *Clitocybe metachroa* is distinguished by its multicolored, more striate, less obviously infundibuliform pileus and longer stipe (32–85 × 3–6 mm). *Clitocybe stercoraria* is separated by its greenish grey basidioma (Bas &al. 1995).

Mycenaceae

Mycena polyadelpha (Lasch) Kühner, Encyclop. Mycol. 10: 262 (1938) Fig. 3

Pileus 0.25–3(–3.4) mm diam., convex to campanulate, surface with fine radial furrows, pubescent, white, slightly transparent, margin somewhat undulating. Stipe (3.5–)4–35(–45) × 0.1–0.2 mm, cylindrical, filiform, hollow, often bent especially when mature, slightly transparent, pruinose to smooth. Flesh very thin, transparent. Lamellae 8(–12), white, distant, deeply adnate and some free.

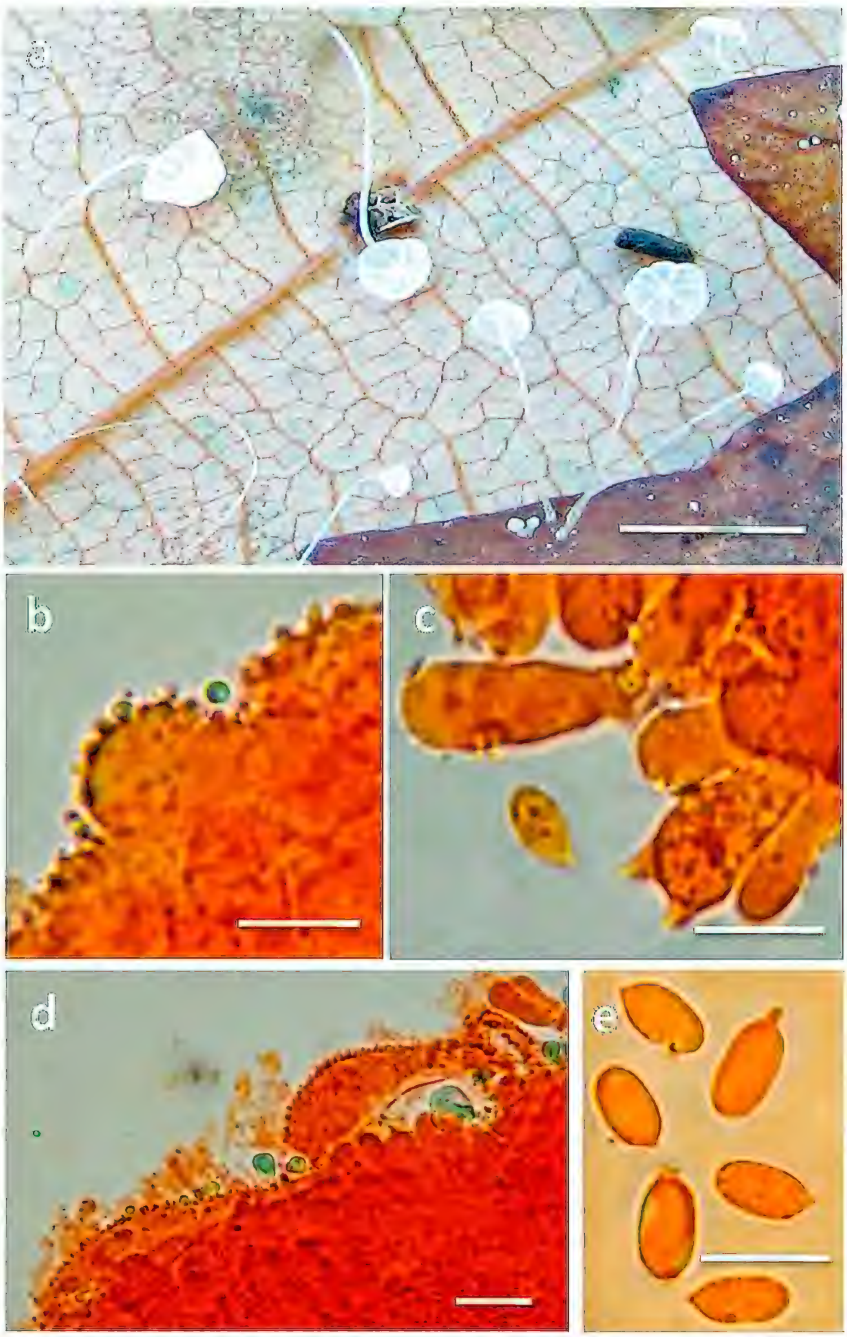
Basidiospores elliptic to cylindric-elliptic (some subglobose), amyloid, (5.3–)6–11 × 3.5–4(–4.9) µm. Cheilocystidia clavate, 18–20(–24.5) × 8–12 µm, roughly warty. Basidia slightly pyriform to cylindric-clavate, (10–)12–15(–15.8) × 5–7.2(–8) µm, 2–4-spored, without basal clamp. Pileipellis hyphae strongly verrucose.

Specimen examined: **TURKEY, Tokat, Akbelen village,** 40°27′51″N 36°39′18″E, 1034 m, on dead leaves of *Quercus* sp. (*Fagaceae*), 4 November 2017, leg. Türkekul & Işik (FGOU 5402).

Comments—The features of our Turkish specimen are compatible with published descriptions of *Mycena polyadelpha* (Moser 1983, Breitenbach & Kränzlin 1991, Gerault 2005, Aronsen 2017).

Mycena polyadelpha belongs to a group of very small mycenas that grow primarily on dead *Quercus* leaves in the winter. *Mycena polyadelpha* resembles *Mycena capillaris* (Schumach.) P. Kumm. and *Mycena mucor* (Batsch) Quél., both of which also grow on fallen *Quercus* leaves. However, *M. capillaris* is distinguished by its adnexed gills while *M. mucor* is separated by its cheilocystidia with long, thin-cylindrical rarely branched excrescences at the apex and stipe with a small basal disc (Breitenbach & Kränzlin 1991, Aronsen 2017).

Fig. 3. *Mycena polyadelpha* (FGOU 5402): A. Basidiomata habit; B. cheilocystidia; C. basidia; D. verrucose pileipellis elements; E. basidiospores. Scale bars: A = 3 mm; B–E = 10 µm.


a
b
c
d
e

Rutstroemiaceae

Rutstroemia conformata (P. Karst.) Nannf.,
Fung. Exsicc. Suec.: no. 1174 (1942) Figs 4, 5

Apothecia 0.8–1.5(–2) mm wide, deeply cup-shaped when young, later disc-shaped, yellowish brown, margin sometimes slightly transparent. Stipe 2–9(–9.5) × (0.2–)0.3–0.45 mm, deep dull brown, thinly white tomentose.

Ascospores (8–)8.5–11.5(–12) × 4–5.5(–6.0) µm, ellipsoid, smooth, hyaline, irregularly uniseriate, usually with two small oil drops (some with one large central drop or several scattered drops), contents otherwise homogeneous. Asci (73–)78.5–110.4 × 6–9.4 µm, cylindric, 8-spored, dull blue at the pore in Melzer's. Paraphyses filiform, septate, branched, hyaline, simple.

Specimen examined: **TURKEY, Tokat, Çamaltı village,** 40°16′13″N 36°26′09″E, 1132 m, on decaying leaves of *Alnus glutinosa*, 27 May 2017, leg. Türkekul & Işik (FGOU 5274).

Comments—Our specimen matched previous descriptions of *Rutstroemia conformata* (Maas Geesteranus 1954, Palmer & Tortic 1994, Verkley 1993).

Rutstroemia conformata resembles *Ciboria amentacea,* which differs by its ochre to ochre-brown hymenium, its non-septate paraphyses, and its much longer stipe (5–25 mm; Breitenbach & Kränzlin 1984).

Fig. 4. *Rutstroemia conformata* (FGOU 5274): A. apothecia in situ. Scale bar = 1 mm.

Fig. 5. *Rutstroemia conformata* (FGOU 5274): B. asci with uniseriate ascospores; C. asci with ascospores and paraphyses; D. branching paraphyses. Scale bars = 10 µm.

Helotiaceae

Symphyosirinia galii E.A. Ellis,
Trans. Norfolk Norwich Naturalists' Soc. 18(3): 11 (1956) Fig. 6

Apothecia (0.5–)1.0–1.5 mm across, shallowly cup-shaped or flat, white or light whitish brown. Hymenium smooth, light cream or pale white. Stipe (3–)5–7(–8) × (0.2–)0.3–0.4(–0.6) mm, smooth, cylindric, white above and becoming brown downward.

Ascospores (9.5–)10–14.6(–15.0) × (3–)3.5–4.0 µm, clavate or slightly scutuloid, hyaline, biseriate, granular, one droplet. Asci (95–)100–127 × 7–8.6(–9) µm, cylindric, 8-spored, arising from simple septa. Paraphyses 90–110 ×1.5–2(–3) µm, filiform to slightly cylindrical, abundant, sometimes forked at the apex.

Specimen examined: **TURKEY, Tokat, Çamaltı village,** 41°27′47″N 36°38′28″E, 1191 m, on fallen mericarps of *Galium* sp. (*Rubiaceae*), 24 September 2017, leg. Türkekul & Işik (FGOU 5362).

Comments—Our Turkish collection has longer stipes than cited by Ellis (1956: 3 × 0.3–0.7 mm) and some ascospores are slightly scutuloid.

Symphyosirinia heraclei E.A. Ellis is distinguished from *S. galii* by its larger apothecia (1–3(–5) mm), its larger ascospores (18–24 × 6–7 µm), and its umbelliferous hosts *Heracleum* and *Conium* (Ellis 1980, Baral 1994). *Symphyosirinia angelicae* E.A. Ellis differs from *S. galii* by its larger apothecia (1–3(–5) mm), its larger ascospores (12–20 × 3.5–5 µm), and its umbellferous hosts *Angelica* and *Peucedanum* (Ellis 1956, 1980; Baral 1994).

Fig. 6. *Symphyosirinia galii* (FGOU 5362): A. long-stiped apothecia; B. apex of asci with ascospores; C. ascospores; D. asci and paraphyses. Scale bars: A = 2 mm; B–D = 10 µm.

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We would like to thank Dr. Abdullah Kaya (Karamanoğlu Mehmetbey University, Karaman-Turkey) and Dr. Ilgaz Akata (Ankara University, Ankara- Turkey) for reviewing this article. We also thank the Gaziosmanpaşa University Research Fund (GOU-BAP: 2012/048) for financial support.

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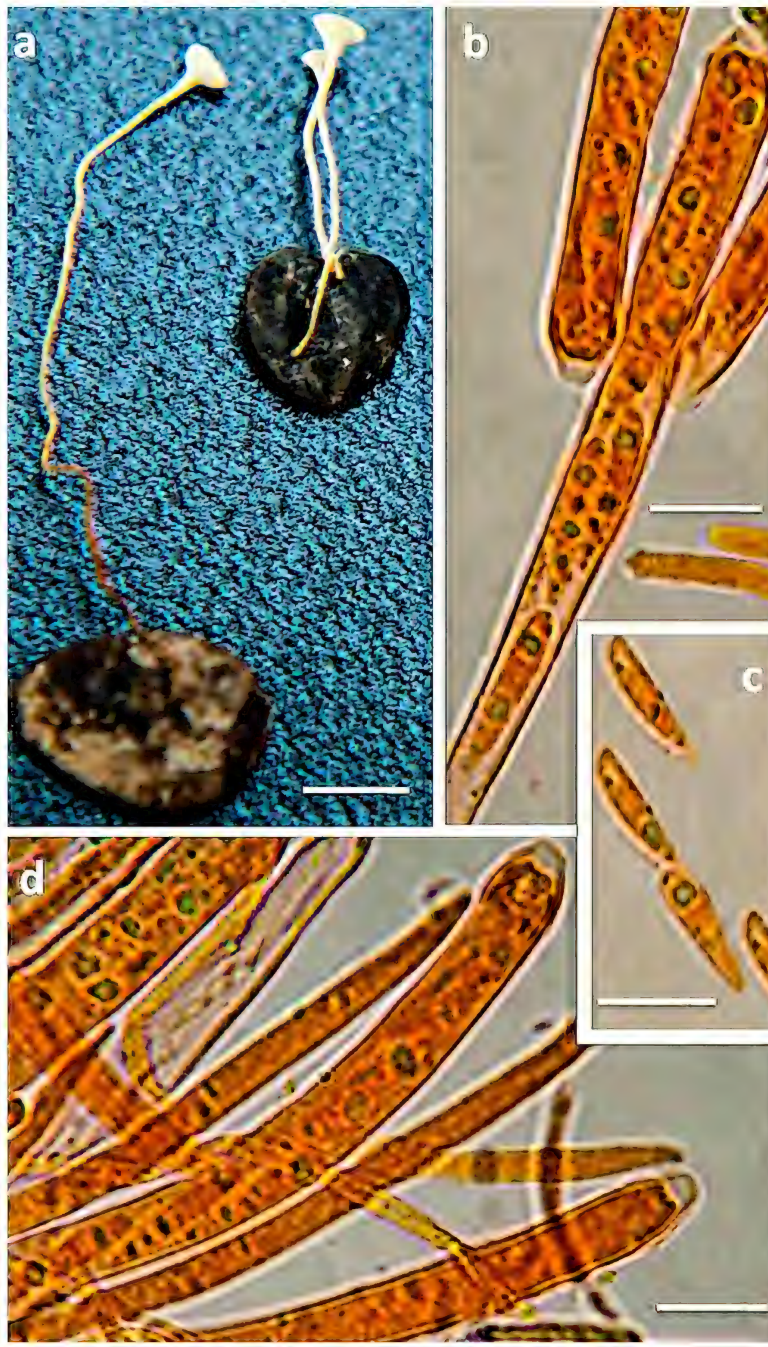
a
b
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d

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Pluteus losulus, a new record from south China

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Abstract—*Pluteus losulus* is reported as a new record from China. It is characterized by its low umbo with brown to grayish-brown pileus, hooked and metuloid pleurocystidia, variable cheilocystidia, presence of caulocystidia, and frequent presence of clamp connections in the pileipellis. New data from the nuclear ribosomal internal transcribed spacer (ITS) region place this species in a sub-basal clade of *P.* sect. *Pluteus*. A morphological description, illustrations, and phylogenetic placement of the Chinese *P. losulus* collection are provided.

Key words—basal clade, *Pluteaceae*, taxonomy

Introduction

Pluteus Fr. sect. *Pluteus* accommodates species with mainly metuloidal hymenial cystidia and a cutis type pileipellis, including the generic type *P. cervinus* (Schaeff.) P. Kumm. [= *Agaricus pluteus* Batsch: Fr.] (Singer 1986; Justo & al. 2011a,b, 2014). In past decades, some taxonomic studies with *Pluteus* distributional records have been reported from China (Teng 1963; 1996; Tai 1979; Bi & al. 1993; Yang & al. 2010, 2011; Xu & al. 2015). However, the fungal resources of this genus have not yet been comprehensively studied for this country.

In an effort to document the *Pluteus* species in China, several collections of *Pluteus* were made in 2017, one of which represented a member of *P.* sect. *Pluteus*. Based on morphological observation and molecular evidence, the examined specimen was identified as *P. losulus*. This species was originally described as *P. cervinus* var. *ealaensis* from Democratic Republic of Congo, Africa, and was elevated to species rank by Justo & al. (2011a) under the replacement name *P. losulus*. Phylogenetically, *P. losulus* was assessed as a basal taxon of *P.* sect. *Pluteus* and sister to *P. atromarginatus* (Justo & al. 2011a). In this study, we describe *P. losulus* as a new record for China. This species is compared here with the basal/sub-basal taxa of *P.* sect. *Pluteus*, and its phylogenetic position is further assessed based on the nuclear ribosomal internal transcribed spacer (ITS) region.

Material & methods

Morphological studies

The specimen was collected from south China (Dinghushan Biosphere Reserve Forest, Zhaoqing City, Guangdong Province, China), described, and deposited in the Fungal Herbarium, Guangdong Institute of Microbiology, Guangzhou, China (GDGM). Macro- and microscopic terminology followed Vellinga (1988).

Macromorphological descriptions were based on the field notes and documented with photographs. Micromorphological observations were made from the dried specimens mounted in H_2O, 5% aqueous KOH (w/v), and Congo Red. Melzer's solution was used to check any amyloid reaction of basidiospores and tissues. Line drawings were freehand. In the descriptions of basidiospore measurements, the notation [n/m/p] was used, which means 'n' basidiospores from 'm' basidiomata of 'p' collections. Dimensions for basidiospores are given as (a–)b–c(–d), in which 'b–c' contains a minimum of 90% of the measured values and extreme values 'a' and 'd' are given in parentheses, when applicable. Q denotes the length/width ratio of a measured basidiospore, and Q_m denotes the average of 'n' basidiospores (presented as mean ± standard deviation).

Molecular studies

Protocols for genomic DNA extraction, PCR amplification, and sequencing followed Hosen & al. (2013) and references cited there. The ITS1-F/ITS4 primer pair (White & al. 1990) was used for the amplification of ITS region.

Representative ITS sequences of *Pluteaceae* (46 samples of *Pluteus* and one sample of *Volvopluteus* Vizzini & al. as outgroup) were retrieved from GenBank for phylogenetic analysis. Sequences were included in phylogenetic analysis based on NCBI blast search results and published molecular analyses (Menolli & al. 2010, 2015a,b; Justo & al. 2011a,b, 2014; Pradeep & al. 2012; Malysheva & al. 2016; Desjardin & Perry 2018), and then combined with the newly generated sequence of the Chinese *Pluteus* collection (GDGM47122). The ITS sequences were aligned in

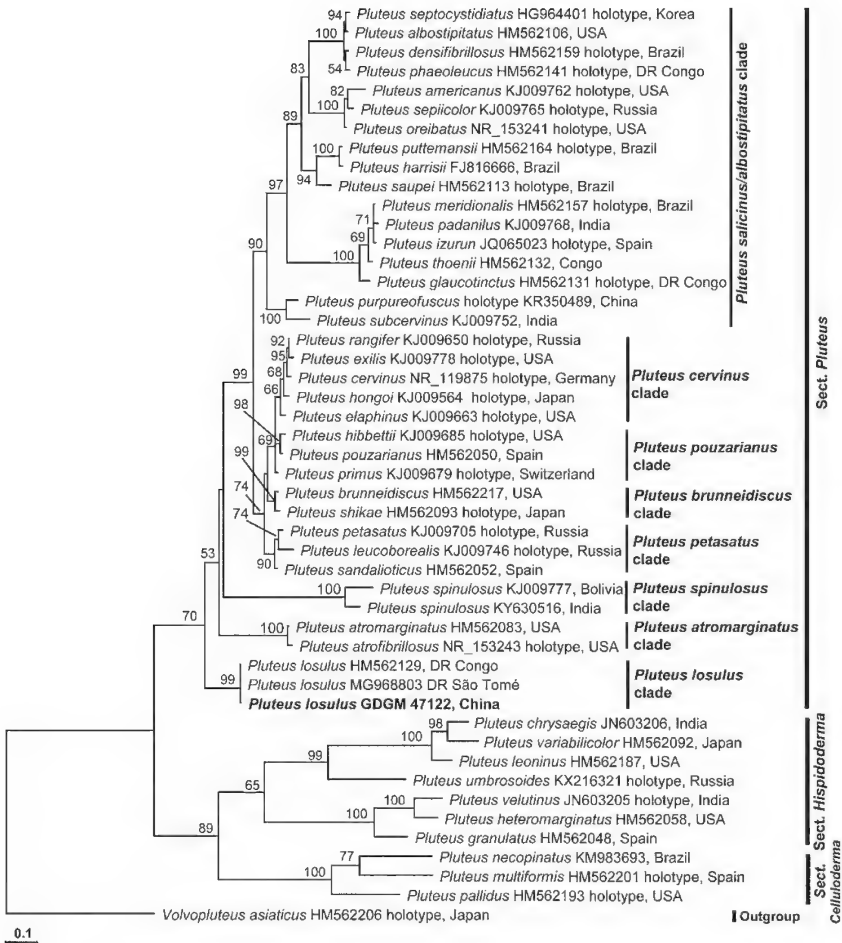
Pluteus septocystidiatus HG964401 holotype, Korea
Pluteus albostipitatus HM562106, USA
Pluteus densifibrillosus HM562159 holotype, Brazil
Pluteus phaeoleucus HM562141 holotype, DR Congo
Pluteus americanus KJ009762 holotype, USA
Pluteus sepiicolor KJ009765 holotype, Russia
Pluteus oreibatus NR_153241 holotype, USA
Pluteus puttemansii HM562164 holotype, Brazil
Pluteus harrisii FJ816666, Brazil
Pluteus saupei HM562113 holotype, Brazil
Pluteus meridionalis HM562157 holotype, Brazil
Pluteus padanilus KJ009768, India
Pluteus izurun JQ065023 holotype, Spain
Pluteus thoenii HM562132, Congo
Pluteus glaucotinctus HM562131 holotype, DR Congo
Pluteus purpureofuscus holotype KR350489, China
Pluteus subcervinus KJ009752, India
Pluteus salicinus/albostipitatus clade
Pluteus rangifer KJ009650 holotype, Russia
Pluteus exilis KJ009778 holotype, USA
Pluteus cervinus NR_119875 holotype, Germany
Pluteus hongoi KJ009564 holotype, Japan
Pluteus elaphinus KJ009663 holotype, USA
Pluteus cervinus clade
Pluteus hibbettii KJ009685 holotype, USA
Pluteus pouzarianus HM562050, Spain
Pluteus primus KJ009679 holotype, Switzerland
Pluteus pouzarianus clade
Pluteus brunneidiscus HM562217, USA
Pluteus shikae HM562093 holotype, Japan
Pluteus brunneidiscus clade
Pluteus petasatus KJ009705 holotype, Russia
Pluteus leucoborealis KJ009746 holotype, Russia
Pluteus sandalioticus HM562052, Spain
Pluteus petasatus clade
Pluteus spinulosus KJ009777, Bolivia
Pluteus spinulosus KY630516, India
Pluteus spinulosus clade
Pluteus atromarginatus HM562083, USA
Pluteus atrofibrillosus NR_153243 holotype, USA
Pluteus atromarginatus clade
Pluteus losulus HM562129, DR Congo
Pluteus losulus MG968803 DR São Tomé
Pluteus losulus GDGM 47122, China
Pluteus losulus clade
Sect. Pluteus
Pluteus chrysaegis JN603206, India
Pluteus variabilicolor HM562092, Japan
Pluteus leoninus HM562187, USA
Pluteus umbrosoides KX216321 holotype, Russia
Pluteus velutinus JN603205 holotype, India
Pluteus heteromarginatus HM562058, USA
Pluteus granulatus HM562048, Spain
Sect. Hispidoderma
Pluteus necopinatus KM983693, Brazil
Pluteus multiformis HM562201 holotype, Spain
Pluteus pallidus HM562193 holotype, USA
Sect. Celluloderma
Volvopluteus asiaticus HM562206 holotype, Japan
Outgroup
0.1

FIG. 1. RAxML tree representing phylogenetic relationships of *Pluteus* species inferred from ITS data. Bootstrap support values ≥50% from Maximum likelihood analysis are indicated on the branches at nodes. *Pluteus losulus* from China is highlighted in bold on the tree. GenBank accession numbers are provided after the species name followed by country of origin. The tree is rooted by *Volvopluteus asiaticus* Justo & Minnis as outgroup.

MAFFT v.6.8 (Katoh & al. 2005), and manually edited in BioEdit v.7.0.9 (Hall 1999) using default settings. Maximum Likelihood (ML) method was used to analyze ITS dataset. ML was performed in RAxML v.7.2.6 (Stamatakis 2006). All parameters in the ML analysis were used as default setting and statistical support values were obtained using nonparametric bootstrapping (BS) with 1000 replicates.

Molecular phylogenetic results

The newly generated ITS sequence of the Chinese *Pluteus* collection was deposited in GenBank (MH231231). In the aligned ITS dataset, sequences of the 48 samples were included with 757 nucleotide sites (gaps included) for each sample. The Chinese collection of *Pluteus* clustered in *P.* sect. *Pluteus* and grouped with African collections of *P. losulus* from Democratic Republic of Congo (GenBank HM562129) and São Tomé, strongly supported by ML analysis (Fig. 1). However, its phylogenetic relationship with other taxa/clade of this section is not satisfactorily resolved. The summarized result of the phylogenetic analysis is presented in Fig. 1.

Taxonomy

Pluteus losulus Justo, Mycol. Progr. 10: 473 (2011). Figs 2, 3

≡ *Pluteus cervinus* var. *ealaensis* Beeli, Bull. Soc. Roy. Bot. Belgique 61(1): 81 (1928).

Basidiomata small to medium sized. Pileus up to 45 mm broad, plano-convex to hemispherical with age, with a low umbo; surface uneven, slightly striate radially, covered with radially arranged fibrils, brown, dull brown, reddish brown or grayish-brown, slightly paler with age; margin entire, slightly sulcate, paler than center. Lamellae 4–5 mm broad, crowded, free, ventricose, pale white when young, becoming pink; lamellulae common, 2–3 tiers, truncate. Stipe 35 × 4 mm, cylindrical to narrowly obclavate, attenuated towards apex, surface covered with whitish to light grayish-brown fibrils; basal mycelium whitish. Context white, unchanged when cut or bruised. Odor and taste not recorded.

Basidiospores [25/1/1] (6.0–)6.5–7.5(–8.5) × 6.0–7.0 µm, [mean 7.11 × 6.40 µm; Q = 1.03–1.16(–1.21); Q_m = 1.11 ± 0.06] nearly globose to subglobose, rarely broadly ellipsoid, hyaline to pale yellowish in KOH and H_2O, smooth, thick-walled. Basidia 20–30 × 8–11 µm, 4-spored, clavate, smooth, thin-walled. Cheilocystidia 23–75 × 10–18 µm, abundant, crowded, variable, clavate to narrowly clavate, subcylindrical, fusoid, sometimes lanceolate to mucronate, hyaline, smooth, thin-walled. Pleurocystidia 60–100 × 15–25(–35) µm, abundant, metuloidal, fusiform to narrowly utriform, sometimes a deformed shape with bifid apices, occasionally with an outgrowth on lateral side, near lamellar edge hooks slightly developed or nearly rounded apices, hyaline. Pileipellis a cutis, terminal elements 75–125(–230) × 7–20 µm, cylindrical, narrowly fusoid to utriform, some strongly tapering towards apex, filled with brown intracellular pigments, smooth, thin-walled. Stipitipellis cutis, vertically oriented, hyphae 4–15 µm wide, cylindrical, pale

FIG. 2. *Pluteus losulus* (GDGM 47122). Basidiomata in natural habitat.

yellowish or colorless, thin-walled, smooth. CAULOCYSTIDIA 40–80 × 12–20 (–25) µm, scattered, fusiform to broadly fusiform, lanceolate, utriform, clavate, with some brown intracellular pigments, thin-walled. CLAMP CONNECTIONS present, common in the pileipellis.

SPECIMEN EXAMINED: **CHINA, GUANGDONG PROVINCE: Zhaoqing City**, Dinghushan Biosphere Reserve Forest, 221 m asl, 12 May 2017, leg. Md. Iqbal Hosen, Hao Huang & Xishen Liang (GDGM 47122, GenBank MH231231).

HABITAT AND DISTRIBUTION—Solitary, found on decayed wood of *Schima superba* Gardner & Champ. (*Theaceae*). Recorded from Democratic Republic of Congo (Justo & al. 2011a), Republic of São Tomé and Príncipe (Desjardin & Perry 2018), and China (this study).

Discussion

The Chinese collection (GDGM 47122) shares a remarkable resemblance with the African material of *P. losulus* described by Justo & al. (2011a), which differed by its larger basidiomata (30–90 mm broad), its longer thicker stipe (35–100 × 3–10 mm), and its partial brown pigmentation in the pleurocystidia; these differences could be due to environmental variation.

The ITS sequence of the African *P. losulus* (Goosens-Fontana 5273) is 99% identical (difference only two nucleotides) to those of the Chinese collection, suggesting that they are conspecific and that the species occurs in two continents. Some other species, e.g., *P. cervinus*, *P. longistriatus* (Peck) Peck, *P. romellii* (Britzelm.) Sacc., *P. velutinus* C.K. Pradeep & al., also have wide geographic distribution (Justo & al. 2011a, Pradeep & al. 2012, Menolli & al. 2015a). However, what dispersal mechanisms (either natural or anthropogenic)

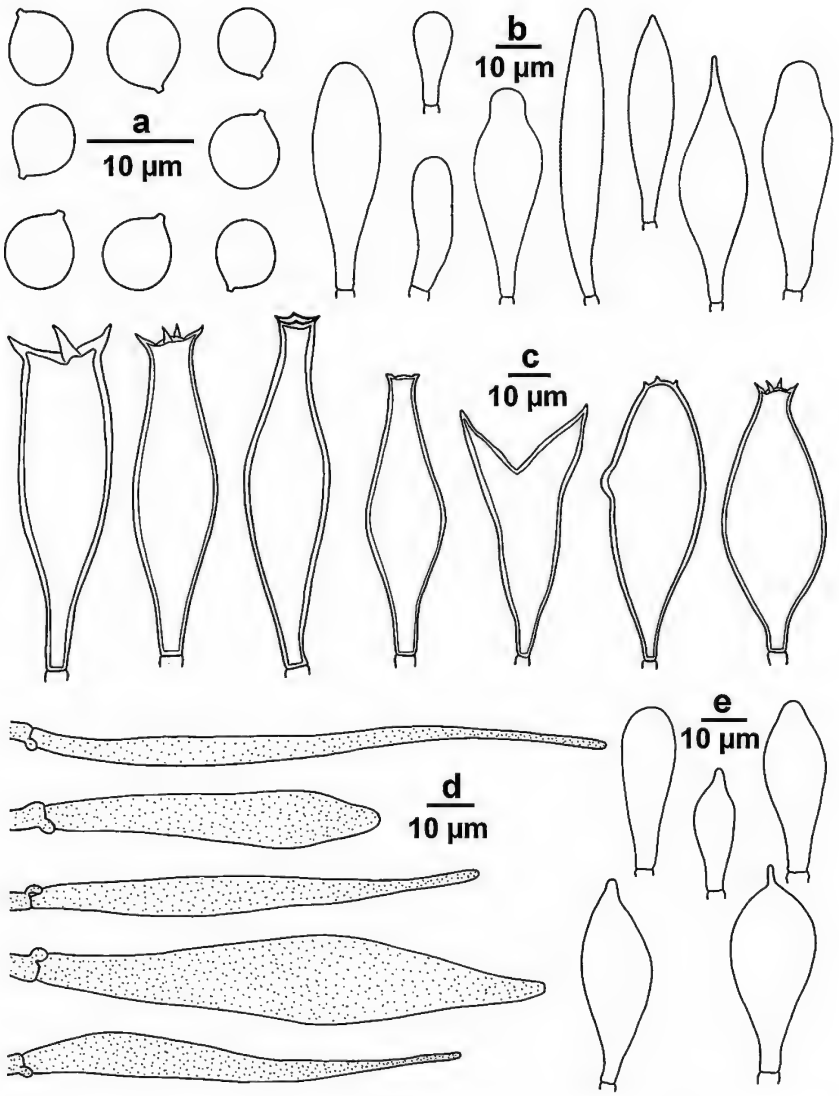

Fig. 3. *Pluteus losulus* (GDGM 47122). a. Basidiospores; b. Cheilocystidia; c. Pleurocystidia; d. Pileipellis elements (terminal cells); e. Caulocystidia.

are responsible for the distribution patterns of these species is still unknown. Gross morphology and molecular data support the identification of the Chinese collection as *P. losulus*.

The presence of clamp connections in *P. losulus* is not surprising, as clamps are found in several species of *P.* sect. *Pluteus*. However, the combination of such features as the hooked and partially pigmented metuloidal pleurocystidia (partial pigment absent from the Chinese specimen), variably sized and shaped cheilocystidia, frequent presence of caulocystidia, and the presence of clamp connections make it distinct within *P.* sect. *Pluteus* (Justo & al. 2011a). *Pluteus losulus* can be compared morphologically with some sub-basal (*P. atromarginatus* (Konrad) Kühner and *P. atrofibrillosus* Vellinga & Justo) and basal (*P. spinulosus* Murrill) taxa. However, *P. atromarginatus* is distinctive in *P.* sect. *Pluteus* because of its relatively large basidiomata (25–100 mm) with fibrillose-squamose pileus, dark brown lamellar edge, very thick-walled (5 µm) pleurocystidia, and association with coniferous trees (Justo & al. 2014); *P. atrofibrillosus* has a densely fibrillose/squamulose pileus, relatively shorter and narrower basidiospores (5.5–7.5 × 4.0–5.5 µm; mean 6.8 × 5.1 µm), and pleurocystidia predominantly with 2 apical hooks (Justo & al. 2014); and *P. spinulosus* is distinguished by its brown basidiomata, usually without apically hooked pleurocystidia, and absence of caulocystidia (Banerjee & Sundberg 1993; Xu & al. 2015).

The molecular study of *Pluteus* by Justo & al. (2011a) showed *P. losulus* together with *P. atromarginatus* as a basal clade in *P.* sect. *Pluteus*. However, in the infrageneric taxonomy of *P.* sect. *Pluteus*, *P. losulus* was separated from *P. atromarginatus* clade, forming an independent clade in which *P. losulus* is placed as the earliest diverging member for this section (Justo & al. 2011b). With the inclusion of ITS sequences of *P. spinulosus* from Bolivia (GenBank KJ009777) and India (GenBank KY630516) that often appear in the phylogeny as a basal clade to *P.* sect. *Pluteus* with moderate BS support, where *P. losulus* has an ambiguous position (preliminary analyses, data not shown) that might be due to lack of significant branch support. In this study, however, *P. losulus* recovered as a basal clade, while *P. atromarginatus* and *P. spinulosus* are poorly supported as close sister clades (Fig. 1). Therefore, the basal clade/taxon of *P.* sect. *Pluteus* remains unclear because neither the *P. spinulosus* clade nor the *P. losulus*/*P. atromarginatus* clade is strongly supported (Fig. 1). In order to establish the ancestral taxon/clade of *P.* sect. *Pluteus* with certainty, more taxa should be sampled from diverse areas.

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Gyalecta caudiospora sp. nov. from China

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Abstract—*Gyalecta caudiospora* is described from China as a new species. It is characterized by its greenish thallus, lecideine apothecia with pale yellow disc, 8-spored ascus, 3-septate ascospores with long attenuated appendage at the lower end, and the absence of lichen substances. The specimens were deposited in LCU. A key to the *Gyalecta* species recorded in China is presented.

Key words—*Gyalectaceae*, lichenized fungi, *Ostropales*, taxonomy

Introduction

Gyalecta was introduced by Acharius in 1808 and has a cosmopolitan distribution on a wide range of substrates (Lücking 1999, Alvarez Andrés & López de Silanes 2002, Gagarina 2011, Aptroot & Moon 2014). The genus is characterized by its crustose thallus, lecideine apothecia, simple paraphyses, 8-spored asci, and transversely septate or submuriform to muriform ascospores.

Gyalecta includes more than 50 species worldwide (Kirk & al. 2008, Baloch & al. 2013, Jaklitsch & al. 2016). Two species: *G. alutacea* Zahlbr. reported from Yunnan (Zahlbruckner 1930, Wei 1991) and *G. foveolaris* (Ach.) Schaer. from Xizang (Obermayer 2004) are known in China. During our study of *Gyalecta*, we collected specimens that differed from previously described species and propose them here as a new species, *G. caudiospora*.

[#] Ming-Zhu Dou & Xiao-Han Wu contributed equally to this manuscript.

Table 1. Absolute distances between alignment sequences of nrLSU region from *Gyalecta caudiospora* and related species.
(Gaps ignored in pairwise comparisons.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *G. caudiospora* MH345767														
2. *G. herculana* FJ941886	44													
3. *G. jenensis 1* KR017187	52	45												
4. *G. jenensis 2* AY340544	51	49	**19**											
5. *G. jenensis 3* AF465450	49	47	**19**	**2**										
6. *G. jenensis 4* AF279391	51	47	**16**	**5**	**5**									
7. *G. friesii 1* KJ766566	61	66	77	71	71	71								
8. *G. friesii 2* HQ659179	61	66	77	71	71	71	**0**							
9. *G. hypoleuca* AF465453	51	43	59	59	59	57	68	68						
10. *G. truncigena* AF465451	60	60	75	76	76	76	48	48	59					
11. *G. thelotremella* AF465455	46	28	53	59	57	57	65	65	32	58				
12. *G. herrei* AF465449	49	19	51	56	54	54	70	70	40	59	28			
13. *G. ulmi* AF465463	59	56	71	68	68	68	26	26	58	50	54	59		
14. *G. leucaspis* AF465462	72	71	83	76	76	76	50	50	71	64	76	74	44	
15. *R. rhexoblephara* KJ766656	68	65	79	76	76	75	51	51	71	64	66	68	43	61

* Infraspecific distances are indicated with bold font.

Materials & methods

Specimens. All materials were collected from Guizhou Province, China, and are deposited in Lichen Herbarium of the College of Life Sciences, Liaocheng University, Liaocheng, China (LCU). Morphological characters were examined and photographed under TECH XTS-30D and Olympus SZX16 dissecting microscopes. The anatomical characters were examined and photographed under an Olympus BX53 compound microscope. Cortex and medulla were tested using K (a 10% aqueous solution of potassium hydroxide), C (a saturated solution of aqueous sodium hypochlorite), and P (a saturated solution of *p*-phenylenediamine in 95% ethyl alcohol). The lichen substances were detected using standardized thin layer chromatography (TLC) (Orange & al. 2010; Jia & Wei 2016).

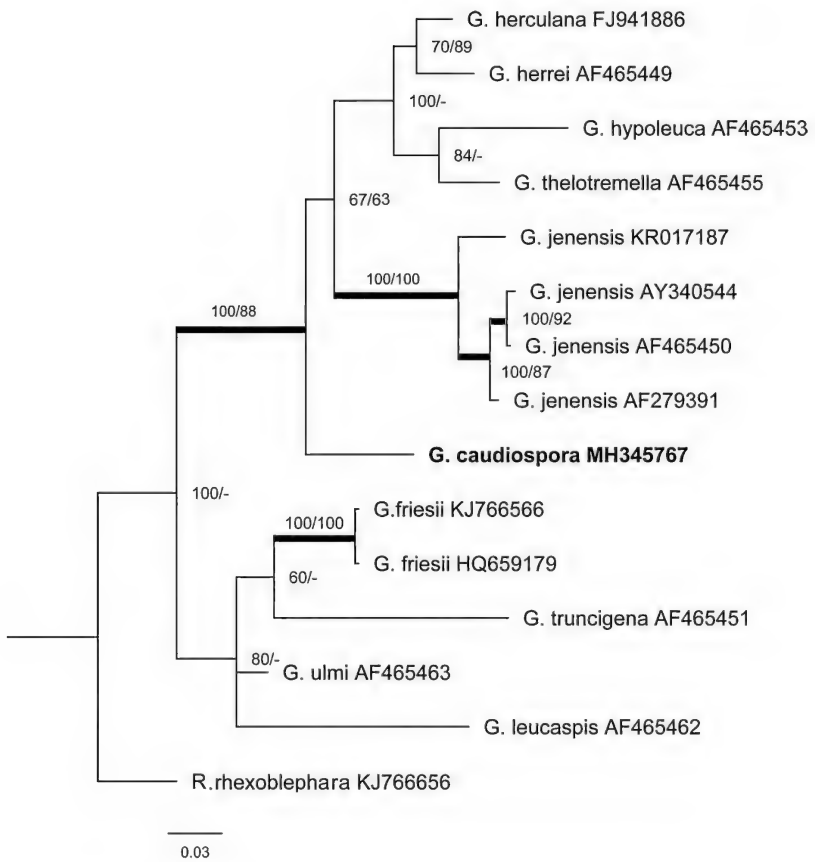

Fig. 1. Phylogenetic relationships inferred from nrLSU sequences of Gyalecta caudiospora and closely related *Gyalecta* species (with Rhexophiale rhexoblephara as outgroup). The evolutionary tree was inferred by using the Bayesian method based on the GTR+I+J model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Posterior probabilities/ML-bootstrap values above 50% are written next to nodes. Branches highly supported by both methods are indicated with a bold line.

DNA extraction, amplification, and sequencing. Genomic DNA was extracted from ascomata of the type specimen using the Hi-DNAsecure Plant Kit according to the manufacturer's protocol. The partial region of nuclear large unit rDNA (nuLSU) was amplified using the AL2R and LR6 primers (Mangold & al. 2008, Vilgalys & Hester 1990) in 25 µL reaction system containing 1 µL each primer solution (10µM), 0.5 µL genomic DNA, 10 µL ddH_2O, and 12.5 µL 2×Taq PCR MasterMix®. Thermocycling conditions comprised initial denaturation at 94°C (5 min); 35 denaturation cycles at

95°C (30 s), annealing at 58°C (30 s), extension at 72°C (1 min), and a final extension at 72°C for 10 min. The target product of PCR was affirmed by electrophoresis on 1% agarose gels and sequenced by Biosune Inc. (Shanghai).

The newly generated sequence was submitted to GenBank and aligned with nuLSU sequences representing similar taxa (Fig. 1). These taxa were selected based on the results of nuLSU sequence Blast searches, morphological characters, and related literature (Baloch & al. 2010).

Phylogenetic analysis and sequence comparisons. The nuLSU sequences from our specimen and 14 selected references were aligned by Muscle using MEGA5 (Tamura & al. 2011). The final matrix can be obtained from the corresponding author.

A phylogenetic tree was generated using Bayesian inference (PP) method based on GTR+I+G model with 6 rates = Invgamma and Maximum Likelihood (ML) method based on the Tamura-Nei model (Tamura & Nei 1993) in MEGA5. The confidence level in the resulting topological bipartitions was estimated with 1000 bootstrap replicates. All alignment gaps and missing data were excluded during phylogenetic analyses via pairwise sequence comparisons. The analyses encompassed 15 nucleotide sequences. Absolute distances were estimated using MEGA5, based on p-distance model with all gaps removed from each sequence pair.

Sequencing & phylogenetic results

The nrLSU partial region from the Guizhou sample was successfully sequenced; the sequence submitted to GenBank contained 953 base pairs. Any sites that were difficult to align were removed from the matrix, reserving 695 total positions for phylogenetic analyses in the final dataset.

Blast searches of nrLSU sequences indicated *G. caudiospora* has close affinities with *G. herrei* (93% identity), *G. herculana* (93% identity), and *G. ulmi* (91% identity). The phylogenetic analyses strongly supported the monophyly of *G. jenesis* (PP = 100%; ML = 100%) and *G. friesii* (PP = 100%; ML = 100%). *Gyalecta caudiospora* did not group with any other species in the tree.

Absolute distances for the aligned sequences of the nrLSU partial region also support the separation of a new species. In our sequence matrix, distances between infraspecific samples are ≤19, while distances between species are ≥19 (Table 1).

Taxonomy

Gyalecta caudiospora Z.F. Jia, **sp. nov.** Fig. 2

Fungal Name FN 570572

Differs from *Gyalecta ancistrospora* by its smaller apothecia, pale yellow and concave disc, and narrower ascospores.

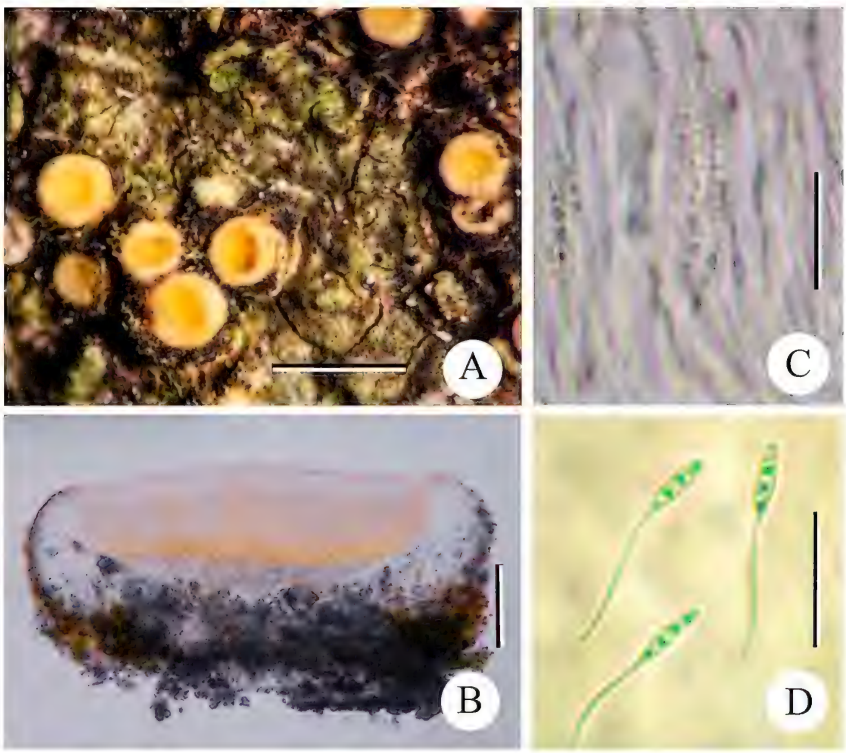

Fig. 2. *Gyalecta caudiospora* (holotype, LCU: X.H. Wu GZ17001). A. Thallus with apothecia; B. Cross section of apothecium; C. Ascus with ascospores; D. Ascospores. Scale bars: A = 1 mm; B = 50 µm; C, D = 20 µm.

Type: China. Guizhou: Xingyi City, Zhengtun Town, Minzu Village, Geyser, 25°06′N 105°06′E, alt. 1240 m, on rock, 1/X/2017, X.H. Wu GZ17001 (**Holotype,** LCU; GenBank MH345767).

Etymology: derived from the Latin *caudatus* + *spora* referring to the long thin appendage on the ascospore.

Thallus crustose, saxicolous, army greenish, dull. Algae trebouxioid, c. 10 × 5 µm. Apothecia lecideine, sessile, 0.15–0.35 mm diam.; disc concave, pale yellow, epruinose, margin pale, higher than the disc; exciple hyaline; epithecium brownish, 8–12 µm tall; hymenium 65–80 µm tall, hyaline, without oil droplets, I+ pale blue, paraphyses simple, 1.5–2.5 µm diam.; hypothecium hyaline. Asci cylindrical to clavate, 40–50 × 7.5–9 µm, 8-spored. Ascospores 25–40 × 2–2.5 µm, 3-septate, fusiform, hyaline, with a long, attenuated appendage at the lower end, c. 1.5–2× the length of the spore body. Pycnidia not observed.

Chemistry: Thallus UV–, C–, K–, KC–, P–. No lichen substances detected with TLC.

Ecology & distribution: On rocks in forest. Known only from the type locality.

Additional specimen examined: China. Guizhou: Xingyi City, Zhengtun Town, Minzu Village, Geyser, alt. 1240 m, on rock, 1/X/2017, X.H. Wu GZ17002 (LCU).

Remarks: *Gyalecta caudiospora* morphologically resembles *G. ancistrospora* Aptroot & K.H. Moon, which differs by its pale greenish grey thallus, its larger apothecia (0.3–0.6 mm) with orange flat disc, and its wider ascospores (3.5–4 µm; Aptroot & Moon 2014).

Both *G. alutacea* and G. *foveolaris* reported in China have smaller ascospores without appendages (Zahlbruckner 1930, Obermayer 2004).

Previous reports that used DNA sequence data to support species delimitation in lichens required both monophyly in phylogeny and diagnostic morphology (Ababaikeli & al. 2016). Our molecular and morphological data of *G. caudiospora* match these requirements for new species recognition.

Key to the species of *Gyalecta* known from China

1. Ascospores small, 5–6 × 1 µm ..... *G. alutacea*
1. Ascospores larger, >10 µm long 2
2. Ascospores 12–16 × 5–6 µm; without appendages ..... *G. foveolaris*
2. Ascospores 25–40 × 2–2.5 µm; with a lower appendage ..... *G. caudiospora*

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Mycobiota (Funga) new to the Mycotaxon website

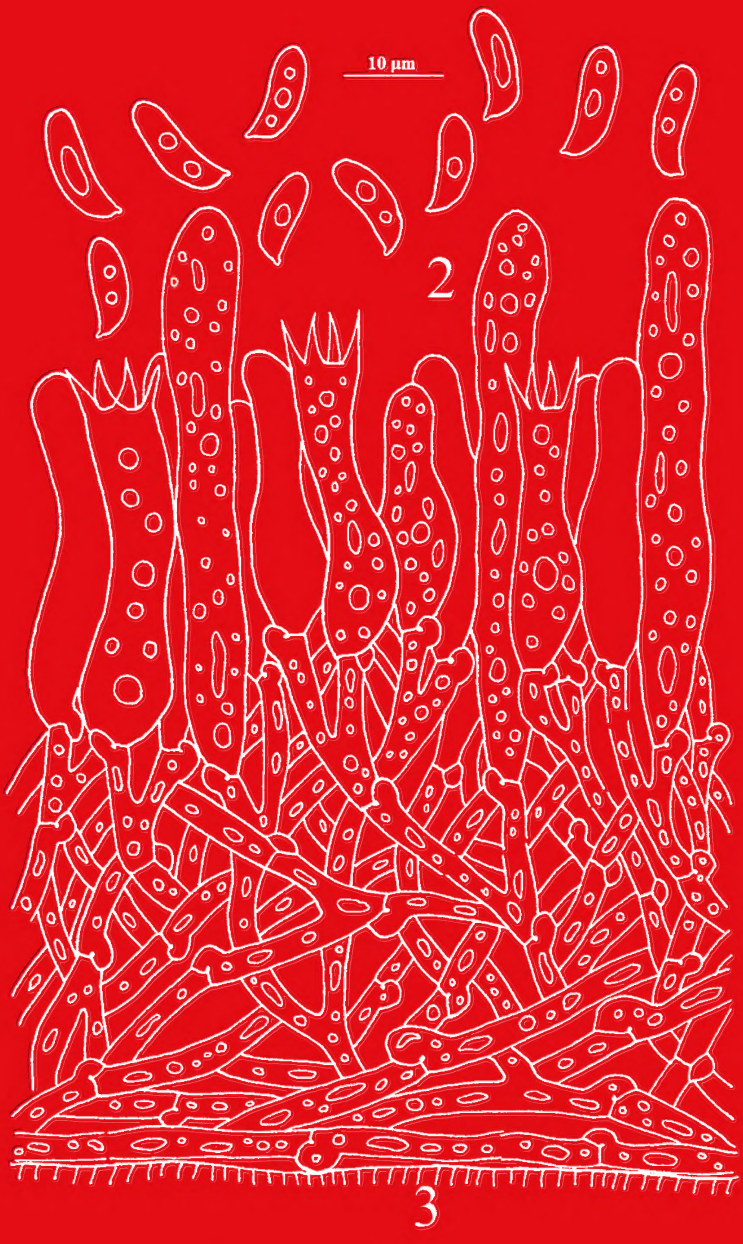
Abstract—Mycotaxon is pleased to add to our 'web-list' page the following new annotated species distribution list under South America (Paraguay): Checklist of the agaricoid fungi from Paraguay by A. Flecha Rivas & N. Niveiro. This brings to 130 the number of free access Fungae now available on our website: http://www.mycotaxon.com/mycobiota/index.html

South America

Paraguay

A. Flecha Rivas & N. Niveiro. Checklist of the agaricoid fungi from Paraguay. 11 p.

Abstract—The aim of this work is to provide a complete literature-based checklist of the agaricoid fungi from Paraguay. For this, an extensive review of the literature and online databases was made. We list 125 species belonging to the agaricoid fungi distributed among 20 families. *Agaricaceae, Tricholomataceae, Psathyrellaceae,* and *Marasmiaceae* are the most species-abundant families. Regarding species distribution, the Capital department has the highest number of reported species, and other biogeographical regions such as Chaco, Cerrado, and Pantanal are practically unexplored.



Sistotrema macrosporum sp. nov.

(Maninder Kaur, Artist.— Figs 2,3, p. 678)